IMMUNOLOGICAL INVESTIGATIONS AND IMMUNOTHERAPY IN LUNG CANCER

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Declaration

In accordance with the regulations of the University of Edinburgh, I declare that this thesis has been composed entirely by myself. I have taken a major part in the work described herein although this has necessarily involved collaboration with others. Their help is acknowledged in the text.

Signed:

ABSTRACT

This thesis examines the value of immunological methods in the treatment, diagnosis and assessment of prognosis of lung cancer.

Delayed hypersensitivity skin tests and laboratory tests of immunological function were performed in patients with operable lung cancer who were then randomly allocated to the autograft or non-autograft groups. In a pilot trial in 15 patients, the autograft group received intradermal injections of autologous irradiated tumour cells and BCG. during three weeks after operation. In this trial only, both groups of patients were given radiotherapy to the mediastinum three weeks after operation. In the subsequent main trial in 83 patients, both groups received one pre-operative percutaneous injection of BCG.. The autograft group only were given serial injections of autologous irradiated tumour cells and percutaneous BCG. during the three weeks after operation.

While the prevalence of positive tuberculin tests among the lung cancer patients before operation was similar to that of controls, sensitisation after challenge by DNCB. was less common in the lung cancer patients, suggesting that there is some impairment of the afferent limb of the immunological response in this condition. Lymphocyte transformation by PPD. but not PHA. or pokeweed mitogen was depressed. Relative depression of certain immunological tests was seen in patients with more advanced disease and a poorer prognosis (total lymphocytes, DNCB.

reactivity) and in squamous cell carcinoma (tuberculin test). The main immunological effect of postoperative immunotherapy was a prolonged increase in tuberculin reactivity.

By constructing actuarial life table curves for survival and duration of freedom from tumour recurrence and by measuring the median times for these, it was shown that DNCB. positive autograft group patients and those with stage I tumours had better clinical results than non-autograft patients ($p = 0.02$ to $p = 0.09$). Although a higher proportion of stage I patients in the autograft group survived free of tumour recurrence two years after operation, the difference was not statistically significant. Adjuvant specific autologous immunotherapy thus seemed, at best, to have only a weak therapeutic action in operable lung cancer.

In a separate study, circulating levels of tumour markers in unselected lung cancer patients were compared with those of control patients with benign pulmonary disease. Elevated levels of carcinoembryonic antigen (CEA.) were found in 17%, of pregnancy-associated α_2-glycoprotein (α_2-PAG.) in 16%, of casein in 14%, of human chorionic gonadotrophin in 6% and of α-foetoprotein in 1.5%. CEA. levels were higher in patients with extensive disease (23%). There was discordance between raised levels of CEA. and α_2-PAG.. Elevated levels of one or more markers were found in 46% of patients in whom four or more markers were measured. In a different series of unselected lung cancer patients, circulating levels of immunoreactive ACTH. were

raised in 24% of patients with small cell carcinoma but only in 3% of patients with non-small cell carcinoma. Elevated levels were commoner in small cell carcinoma patients with extensive disease.

The results showed that when the upper limit of "normal" was that of patients with benign pulmonary disease, the prevalence of elevated levels of tumour markers was much lower than that claimed by other authors. Hence measurement of these markers is of little or no diagnostic value in lung cancer.

IMMUNOLOGICAL INVESTIGATIONS AND IMMUNOTHERAPY IN LUNG CANCER

Introduction

During the past 10 years, I have been a member of a team of medical scientists who have been active in research on lung cancer (the West of Scotland Lung Cancer Group). As a clinical member, I have been closely involved in a number of immunological investigations into the diagnosis and treatment of this condition. This thesis is an attempt to summarise this work and to combine our experience with that of other workers in order to describe the present state of knowledge in this interesting and expanding field.

CHAPTER 1

EVIDENCE OF IMMUNOLOGICAL DEFENCE AGAINST CANCER

The use of immunological techniques in the diagnosis and treatment of cancer is based on the belief that there is a host defence mechanism against tumours and that this includes the production of antibody and the activation of specialised immunological cells. The reasons for this belief can be summarised under the following headings:

1. Variable progress and occasional spontaneous regression

Clinical experience has shown that histologically similar tumours have widely varying growth rates in different individuals. This has recently been confirmed by Geddes (1979) who measured tumour doubling times in patients with bronchial carcinoma. Occasional spontaneous regression of histologically proven tumours has been reported. For example, Everson and Cole (1956) described this occurrence in 47 patients collected from the literature during this century. They included one case of lung cancer (Blades and McCorkle, 1954). Moreover the occurrence of metastases many years after successful treatment of a primary carcinoma is an occasional feature of some tumours e.g. breast (Stewart 1952). This would suggest that cells deposited in a distant organ at or before the time of operation can remain there over years prevented from further growth by the host defence mechanism. In this context we should also add the poor relationship between the pathologist's estimate of "grade of malignancy" and the clinical course of cancer (Southam, 1960).

2. "Cure" following incomplete removal of tumour

Surgeons are sometimes surprised to find that patients in whom only palliative resection of tumour has been achieved live indefinitely without further progress of tumour. This has been described in gynaecological tumours by Graham and Graham (1955) and in lung cancer by Abbey Smith (1970). This would suggest that natural defence mechanisms are capable of eliminating small populations of cells from the body. That this mechanism may be immunological in nature is suggested by the occurrence of tumours in patients who are taking immunosuppressive drugs e.g. following kidney transplantation (Penn, 1978). These drugs may depress the immunological surveillance mechanism so preventing the recognition of small numbers of tumour cells as foreign.

3. Invasion of tumours by cells normally immunologically active

Invasion of tumour tissue by lymphocytes, plasma cells and macrophages has been widely reported. In general these cells are more abundant in well differentiated lung cancers than in small cell carcinomas and may be seen in relation to destroyed tumour cells (Ioachim, Dorsett and Paluch, 1976). Plasma cells are especially numerous in squamous cell carcinoma.

Immunology of Lung Cancer

Immunotherapy is based on the belief that there is an immunological defence against tumours and this can be increased by immunotherapeutic procedures. Before discussion

of immunotherapy in lung cancer, it is necessary to consider the evidence that this tumour can evoke an immunological response. This evidence can be summarised under the following headings:

1. Existence of lung cancer associated antigens.
2. Circulating antibodies to these antigens.
3. Immune complexes.
4. Relationship between active cell-mediated immunity and prognosis.

1. Lung Cancer Associated Antigens

In order to demonstrate the existence of tumour-associated antigens in lung cancer, antisera have been prepared by injecting 1.3 - 3M KCl extracts of tumours into laboratory animals (Frost, Rogers and Bagshawe, 1975). These antisera have then been absorbed with normal lung and other body tissue components. Precipitin formation after double immunodiffusion in agarose or, alternatively, indirect immunofluorescence in tissue sections (Bell and Seetharam, 1976) have been used to detect the presence of antigen.

A variation of this method is the active immunisation of rabbits with extracts of human lung cancer together with rabbit antibody raised against normal human lung extract. The latter step inhibits formation of antibodies against normal human lung tissue. After a single absorption with normal lung tissue extract, the antigen obtained is highly specific for the histological type of tumour (Kelly and Levy, 1980).

Antigens of molecular weight between 40 and 200 x 10^3 daltons have been isolated (Braatz, McIntire, Princler et al. 1978; Gennings, Leake and Bagshawe, 1979). One antigen isolated had a sedimentation coefficient of 7S and an electrophoretic mobility in the β_2 globulin range (Sega, Citro and Natali, 1979). Glycoproteins and lipoproteins have been among the antigens described.

It is well known that antigens to which animals are exposed before or shortly after birth can suppress any future reaction to such antigens when the immunological system has reached maturity (Burnet and Fenner, 1949). The fact that human cancer cells, including those of lung cancer, carry antigens which also react with antisera raised against foetal antigens may explain why the immunological defence against these cells is ineffective. Watson, Smith and Levy (1975) described two antigenic components of lung cancer. Antiserum to the first reacted with normal foetal lung and had relatively low cross reactivity with histologically similar tumours. Antiserum to the second did not react with foetal lung but had higher cross reactivity with tumours of the same and other histological types. Ford and Newman (1979) found that antiserum raised against small cell carcinoma also identified antigens present in normal and foetal lung tissue. Hollinshead and Stewart (1977) identified as many as 5 antigens on squamous cell carcinoma, 4 of which cross-reacted with foetal tissue. There is thus antigenic similarity between immature tissues whether they be foetal or neoplastic.

Antiserum against one histological type of lung cancer will react with extracts of other histological types and even with extracts of tumours of other organs, though with less frequency (Kelly and Levy, 1977). However, while cross-reactivity between squamous cell carcinoma and adenocarcinoma is common, antisera raised against plasma membrane antigen of small cell carcinoma do not react with extracts of other histological types (Bell and Seetharam, 1976). More recently the National Cancer Institute group, using affinity chromatography and polyacrylamide gel electrophoresis, have isolated a highly specific human lung tumour-associated antigen. Tumour extracts from 85% of lung cancer patients but only 8% of patients with other carcinomas gave a positive reaction (Herberman, McIntire, Braatz et al., 1978). Using enzyme-linked immunoassay (ELISA) Kelly and Levy (1980) were able to show that sera from stage I lung cancer cases inhibited the interaction of specific antitumour antibody with human tumour-associated antigen preparations. Normal control sera did not inhibit the interaction.

Most tumour-associated antigens described are surface antigens and can be isolated from cultures of lung cancer cells (Cerni and Micksche, 1976) and from malignant pleural effusions (Cannon, McCoy, Dean et al., 1977; Herberman et al., 1978). There is also indirect evidence of tumour antigens in thoracic duct lymph (Han and Takita, 1976).

Extracts of autologous and allogeneic tumour tissue (Boddie, Holmes, Roth and Morton, 1975), and cell culture

lines of squamous cell carcinoma and malignant pleural effusions (McCoy, Jerome, Cannon et al., 1977) can inhibit migration of peripheral blood leucocytes from lung cancer patients. This effect is not specific for leucocytes taken from patients with the same histological type of lung cancer and does not vary with the stage of the tumour (Cannon et al., 1977). It can be enhanced by washing the leucocytes to remove adherent "blocking factors", (Marabella, Takita, Takada and Minowada, 1975).

Plasma membrane fractions of different histological types of lung cancer have been shown to inhibit adherence of leucocytes from patients with lung cancer. This occurred in 80% of patients with the same histological type of tumour, 24% of patients with lung tumours of different histology but in only 14% of patients with other chest diseases (Anthony and Millband, 1978).

Tumour extracts can also stimulate protein synthesis in lymphocytes from lung cancer patients (Roth, Holmes, Boddie and Morton, 1975) and can inhibit mitogen-induced transformation of lymphocytes from normal donors (Roth, Chee, Morton and Holmes, 1978).

Serum from lung cancer patients also inhibits migration of peripheral blood leucocytes and blocks mitogen-induced transformation of lymphocytes. The latter has been shown using normal lymphocytes from human donors (Roth et al., 1978); and rabbit mononuclear cells stimulated by bacterial extracts (Kubickova, Kubin, Svejcar, et al., 1979).

Although the exact factors responsible for the inhibitory action of lung cancer serum is not known with certainty, they may include tumour antigens. While these may be bound in immune complexes in the serum, the inhibitory action of tumour extracts and thoracic duct lymph suggest that immune complex formation is not essential for inhibition.

2. Antibodies: presence in lung tumour tissue and the circulation

There are relatively few reports of the presence of antibodies in host tumour tissue. This may be because they are produced intermittently in small amounts or because they are combined in antigen-antibody complexes (Paluch and Ioachim, 1978). Low antibody production may arise because the antibody-producing cells are inhibited by tumour factors or because tumour antigen evokes only a cellular response (Kennel, 1979).

Antibodies to lung cancer cells have been demonstrated in serum, tumour extracts, malignant pleural effusions and bronchial washings but only infrequently and with great difficulty. In an attempt to demonstrate antitumour antibodies in serum from lung cancer patients, Takada, Takita and Marabella (1976) added test serum to cultured cells from allogeneic small cell carcinoma and adenocarcinoma of lung and from carcinoma of the cervix. The cells were then stained using an immunofluorescent technique. Evidence of antitumour antibody was found in serum from only one of 26 patients. Complement-dependent cytotoxic

antibody was found in sera from 3/18 patients by Dawson and Moore (1975). Sera from 8/18 patients induced cellular cytotoxicity in leucocytes from healthy donors. The same authors found that lung cancer patients' sera showed blocking activity against allogeneic leucocytes from lung cancer patients. Using an indirect immunofluorescent technique Gorny, Jezewska, Krzysko et al. (1979) detected antibody against allogeneic squamous cells in culture in 22%, against autologous cultured cells in 50% and against autologous and allogeneic fresh squamous carcinoma cells in 66% of sera from lung cancer patients. They found that these sera contained specific IgM antibodies against surface antigens.

Antibodies have been detected in tumour tissue by Paluch and Ioachim (1978). Acid eluates from minced squamous cell and adenocarcinoma tissue were found by radial immunodiffusion to contain mainly IgG with small amounts of IgA and IgM. Eluates from small cell carcinoma contained minimal amounts of IgG but no IgA or IgM. Immunoglobulins eluted from tumour tissue and pleural effusions of squamous cell carcinoma and adenocarcinoma were shown by immunofluorescence to react with tissue cultures and suspensions of cells of the same histological types. This evidence that immunological activity is greater against squamous cell carcinomas and adenocarcinoma than against small cell carcinoma supported the observation of the same workers that a local mononuclear cell reaction is a feature of squamous cell carcinoma and adenocarcinoma

rather than of undifferentiated carcinomas (Ioachim, Dorsett and Paluch, 1976).

B lymphocytes are the progenitors of the differentiating line of cells whose mature member is the plasma cell. Increased numbers of B lymphocytes have been found in patients with squamous cell carcinoma that has spread to local lymph nodes, (Ritts, Jacobson, Caron et al., 1977). A relative increase of circulating B cells also occurred with tumour progression (Anthony, Kirk, Madsen et al., 1975). However, increase in B cell concentrations in the circulation has not been widely reported in lung cancer.

As all antibodies are immunoglobulins, changes in the serum levels of these might give some clue to the ability of the humoral defence mechanism to resist tumour cells. Plesnicar and Rudolf (1979) found that survival times of patients with lung cancer were longest when the levels of individual serum immunoglobulins fell within a narrow range towards the upper limit of normal.

It may thus be that there is an optimum level of circulating antibody. Below this level, the circulating antibody is insufficient to neutralise tumour antigen. Above it, excess antibody blocks the antitumour activity of immunocompetent cells. However other factors, e.g. the distribution of surface antigen receptors on the tumour cells, also govern the effectiveness of antitumour antibody.

3. Immune complexes

In 1971, Sjögren, Hellström, Bansal and Hellström reported that sera from mice bearing experimentally induced sarcomas blocked the cytotoxic effect of lymphocytes immune to the relevant tumour specific antigen. This blocking factor consisted of two components, one of high and one of low molecular weight, neither of which had blocking activity in a standard test by itself. They postulated that the blocking activity was due to circulating antibody-antigen complexes.

In an extensive review of circulating immune complexes in cancer, Baldwin and Robins (1980) classified methods of measuring these into four groups:

Physical separation of complexes

Interactions with complement e.g. C1q binding

Interactions with rheumatoid factors

Interactions with cells e.g. Raji cell binding and Staphylococcus aureus binding.

Tests of C1q binding measure complement binding immune complexes whereas the Raji cell and the Staphylococcus aureus binding technique measure both complement and non-complement binding immune complexes.

Circulating immune complexes in cancer have sedimentation coefficients between 10 and 30 S and can be dissociated at low pH into IgG fractions reacting in membrane immunofluorescence tests with tissue culture cells (Paluch and Ioachim, 1978).

Using Raji cells, a human lymphoblastoid cell line

with B cell characteristics, Theofilopoulos, Wilson and Dixon (1976) demonstrated circulating immune complexes in 2 of 7 lung cancer patients tested. Evidence of circulating immune complexes using the C1q binding assay was found in 90% lung cancer patients by Rossen, Reisberg, Hersh and Gutterman (1977) and in 67% by Heier, Carpentier, Lange et al. (1977). However, using the same technique, Lowe, Segal-Eiras, Iles and Baldwin (1981) found increased C1q binding activity in only 34% of lung cancer patients and in as many as 20% of age and sex matched controls. There was no significant difference in mean values between patients and controls although the highest levels were seen in the lung cancer patients. Absence of correlation with total wbc. count and ESR, was taken to indicate that increased C1q binding activity was not due to infection. This is a questionable assumption as neither of these are very reliable guides to infection in lung cancer; the wbc. can be considerably elevated in patients with rapidly growing tumours in the absence of infection. Serum C1q binding activity was not related to histological type or survival but did correlate with the extent of malignant disease. This confirmed the finding of Jansen, The, de Gast et al., (1977) who also found a higher level of circulating immune complexes in patients with large and small cell undifferentiated carcinomas.

More recently, Guy, di Mario, Irvine et al. (1981) found elevated serum C1q binding (solid phase) in only 13% of lung cancer patients compared with 10% of normal blood donors and 6% of bronchitic patients. Using the

Raji cell and Staphylococcus aureus binding techniques, these authors found elevated levels in 44% and 33% respectively of lung cancer patients compared with 50% and 44% of bronchitic patients. Although bronchitic patients with active infection were excluded, previous infection may well have been a factor in causing elevated levels in both groups of patients. Indeed these authors found no evidence that immune complexes in lung cancer patients contained a tumour specific antigen component.

Measurement of immune complexes by existing techniques is of little or no diagnostic value but it might prove useful in monitoring progress of the disease and the response to therapy (Lowe et al., 1981).

4. Immunological reactivity and prognosis

One of the earliest discoveries during the investigation of immunological changes in lung cancer was that patients with positive delayed hypersensitivity skin (DHS.) tests and/or active cell-mediated immunity survived longer than those without. Patients with positive delayed hypersensitivity skin reactions to tuberculin (Israel, Bouvrain, Cros-Decam and Mugica, 1968) and dinitrochlorobenzene (DNCB.) (Krant, Manskopf, Brandrup and Madoff, 1968) had significantly greater survival times than negative reactors. In addition, a higher proportion of patients with impaired lymphocyte reactivity to phytohaemagglutinin (PHA.) died within 2 months than of those with normal lymphocyte reactivity (Han and Takita, 1972).

The prognostic value of delayed hypersensitivity skin reactions has since been confirmed for recall antigens including tuberculin (Israel, Mugica and Chahinian, 1973) and for new antigens including DNCB. (Inoue, Ishihara, Kobayashi, and Fukai, 1978; Liebler, Concannon, Magovern et al., 1977). Moreover, as T lymphocytes are an essential component of the cell-mediated immunological reaction, the relationship between circulating T cell levels and prognosis has been studied. Stefani and Kerman (1979) found a linear relation between total circulating T cells and survival. In the study of Dellon, Potvin and Chretien (1979), all patients with an absolute T cell count less than 750/ml died or developed metastases within 9 months. In contrast 55% of patients with pretreatment T cell levels more than 750/ml were alive and free from tumour metastases 9 months after treatment.

The position with lymphocyte reactivity to mitogens is not so clear cut. Most other studies have confirmed that normal lymphocyte reactivity is associated with a better prognosis than depressed reactivity (Wanebo, Rao, Miyazawa et al., 1976; Liebler et al., 1977; Giuliano, Rangel, Golub, et al., 1976). However, Barnes, Farmer, Penhale et al. (1975) found no difference in survival of patients with disseminated and anaplastic tumours after operation between those with normal and those with depressed lymphocyte reactivity to PHA. Similarly Braeman and Deeley (1973) found no relationship between

lymphocyte reactivity to PHA. and purified protein derivative of tuberculin (PPD.) and survival after irradiation of tumour cells. This discrepancy might be explained by the combination of surgery and advanced stage of the tumour in the first series and the long standing depression of immunological activity by radiotherapy in the second.

Patients with strong delayed hypersensitivity skin reactions and normal cell mediated immunity have a better prognosis than those without; this suggests that lung cancer evokes an immunological defensive response in the host. However, there are 3 other possible explanations:

1. Cell-mediated immunological reactions are depressed in proportion to the size and extent of tumour tissue.
2. Patients with normal immunological reactivity respond better to treatment.
3. Malnutrition, a feature of more advanced cases, depresses cell-mediated immunity.

Tests of delayed skin hypersensitivity and cell-mediated immunity are depressed in patients with advanced tumours. This has been shown for skin reactions to DNCB. (Wanebo et al., 1976; Giuliano et al., 1979) and tumour extracts (Weese, Herberman, Hollinshead et al., 1978), for absolute numbers of circulating T cells (Shirakusa, Shigematsu and Yoshida, 1978) and for lymphocyte responsiveness to mitogens (Wanebo et al., 1976; Giuliano et al., 1979). If it were caused solely by factors released by the tumour, depression of these tests in an individual might simply

indicate that the tumour was advanced and likely to cause death soon. However even in series of patients with maximal tumour burdens such as the inoperable cases of Han and Takita (1972) and the Stage 3 small cell carcinoma cases of Jansen, Esselink, Orie and The (1979), active cell mediated immunity carried a relatively better prognosis. Moreover, patients with metastases but normal skin and laboratory tests had as good a prognosis as those with localised disease but abnormal tests (Liebler et al., 1977).

Patients with evidence of active cell-mediated immunity respond better to chemotherapy (Pouillart, Schwarzenberg, Huguenin et al., 1976) and radiotherapy (Stefani and Kerman, 1979) and are more likely to have operable tumours (Inoue et al., 1978). However, even in patients who have completed therapy, normal cell-mediated immunity carries a better prognosis (Gross and Eddie-Quartey, 1976). Thus response to treatment is not the only factor linking the results of immunological tests with prognosis.

Malnutrition depresses delayed hypersensitivity skin tests and cell-mediated immunity. As this is a late feature in lung cancer, it would not account for the association of depressed immunological reactivity with a worse prognosis in patients with resectable tumours (Inoue et al., 1978).

It is thus evident that positive delayed hypersensitivity skin tests and active cell-mediated immunity indicate that lung cancer can evoke an immunological response. This response can help resistance to the progress of the tumour and so increase survival.

CHAPTER 2

IMMUNOLOGICAL MECHANISMS OF DEFENCE AGAINST TUMOUR CELLS

In order to understand the possible role of immunotherapy in lung cancer, we need to consider some of the immunological mechanisms which may be involved in the defence against tumour cells, why these have failed in patients with established tumours and how this failure might be corrected.

The principal cells involved in the response to tumours are lymphocytes and macrophages and their roles are summarised in Figure 1. Those cells which actually damage tumour cells are:

1. Lymphocytes
   (a) T lymphocytes
   (b) Antibody-determined killer (K) cells
   (c) Natural killer (NK) cells
2. Macrophages

Lymphocytes

The ability of lymphocytes to damage tumour cells has been shown in numerous studies of "cytotoxicity". In these studies lymphocytes from peripheral blood are added to cultured tumour cells. Cell viability can be assessed by staining since killed cells stain with trypan blue or crystal violet whereas living cells exclude these dyes (Hellström, Hellström, Sjögren and Warner, 1971; Konda and Smith, 1973). Viability of target cells can also be assessed by the release of radioisotope with which they have been labelled or by the uptake of certain vital metabolites labelled with radioisotopes. Schecter, Treves and Feldman (1976) showed decreased uptake of ^{3}H leucine and ^{3}H thymidine by

target Lewis lung tumour cells from mice exposed to autologous lymphocytes. More recently, Vose, Vanky, Fopp and Klein (1978) found evidence of cytotoxicity against ^{51}Cr-labelled autologous lung tumour cells in peripheral blood lymphocytes from 15 of 47 patients with lung cancer. Cytotoxicity against allogeneic lung cancer and other cancers was rare.

Other indirect evidence of lymphocyte involvement has come from *in vivo* studies. Konda and Smith (1973) observed an increase in lymphocyte population of spleen and lymph nodes of mice in whom methyl cholanthrene-induced sarcomas had been produced. In humans Ioachim *et al*., (1976) noted a marked infiltration of lymphocytes and plasma cells in relation to destroyed tumour cells in lung cancer tissue.

It is likely that at least some T lymphocytes are cytotoxic to tumour cells. Konda and Smith (1973) found increased T cells in their tumour bearing mice. Janik and Szarniawska (1978) found that T lymphocytes were necessary for restoration of immunity to transplanted tumour in irradiated thymectomised mice. Using autologous target cells from freshly prepared lung tumours, Vose (1980) showed that in 9 of 21 patients where cytotoxicity was demonstrated, the effector cells were mainly T cells. Moreover Ramey, Hashim, Munther *et al*. (1980) found evidence of lung tumour antigen-sensitive T cells in 20/32 lung cancer patients.

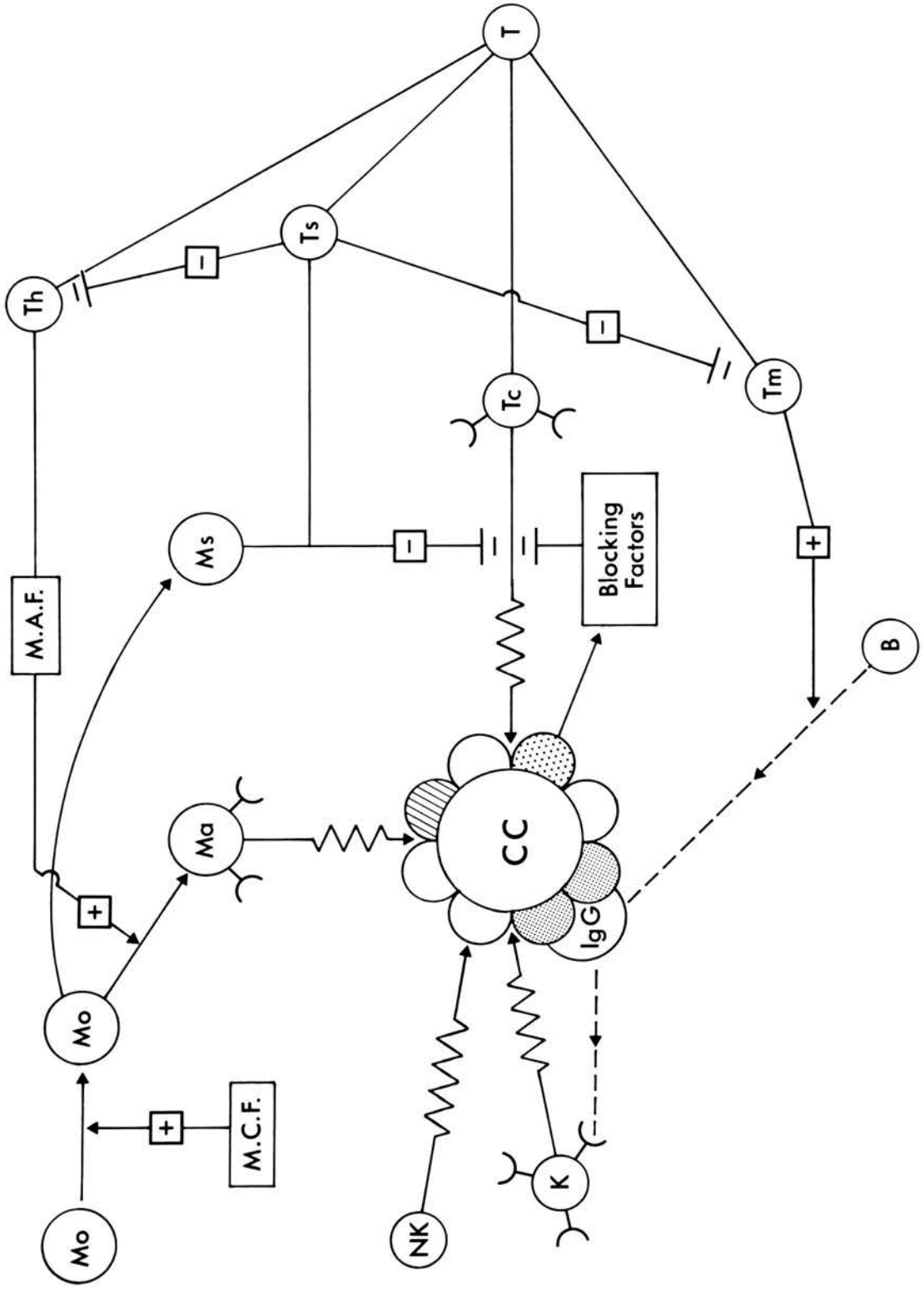

Fig. 1 Possible immunological mechanisms involved in defence against the cancer cell. See legend overleaf.

Fig. 1. Immunological mechanisms involved in the defence against tumour cells. The diagram shows a cancer cell (CC) coated with different antigens being attacked by a cytotoxic lymphocyte, (Tc), an activated macrophage (Ma), a killer cell with antibody receptors (K), and a natural killer cell (NK). The stem T cell (T) gives rise also to helper T cells (Th), helper cells with receptors for IgM (Tm), and non-adherent suppressor T cells which inhibit other lymphocytes (Ts). B cells (B) lead to production of antibody (IgG) attached to antigen on the cancer cell surface.

Monocytes (Mo) aggregate under influence of monocyte chemotactic factor (M.C.F.) and become activated under influence of macrophage activating factor (M.A.F.). They also form adherent suppressor cells (Ms) which inhibit the cytotoxic action of T cells. Blocking factors, possibly immune complexes, also have this action.

The role of killer (K) cells in lung cancer has not yet been well defined. These are lymphocytes which possess surface receptors for IgG and which are capable of binding to and damaging tumour cells coated with this immunoglobulin (Jonsdottir, Dillner-Centerlind, Perlmann and Perlmann, 1979). While some of these cells possess T cell markers, others lack surface markers of B and T cells and are therefore "null cells".

There has recently been considerable interest in another group of cytotoxic lymphocytes, the natural killer or NK cells (Herberman, Timonen, Ortaldo et al., 1980; Mitchison and Kinlen, 1980). Most NK activity has been found among large granular lymphocytes with indented nuclei. NK cells make up 1 - 2% of all lymphoid cells in spleen or peripheral blood. In vitro experiments have shown that they are spontaneously cytolytic not only for tumour cells but also for a variety of cells infected by viruses. In vivo they increase the rate of clearance of labelled tumour cells. NK cells have surface receptors for IgG but IgG does not inhibit their killing ability. The possession of some T cell surface markers suggests that NK cells are related to the T lymphocyte series but they do not form E-rosettes under the usual laboratory conditions and are thus also "null cells". It is of particular significance that NK cell activity is enhanced by Bacille Calmette Guérin (BCG.) and Corynebacterium parvum (C. parvum) (also by interferon) and that NK activity decreases with age.

As well as having a direct cytotoxic action, T lymphocytes also act on two other groups of cells, B lymphocytes and macrophages.

With the development of techniques using mouse monoclonal antibodies it has become possible to identify two contrasting subsets of T cells (Janossy, Tidman, Selby et al., 1980). Helper T cells (designated Tm cells because they bear surface receptors for IgM) react with mouse monoclonal antibody OKT_4. They help B cell proliferation and differentiation into plasma cells (Moretta, Mingari, Moretta et al., 1980) and lymphocyte response to PHA. and Con A. This helper activity is resistant to irradiation but may be reduced in patients with stage 3 lung cancer (Jansen, The and Orie, 1979). In contrast, suppressor T cells (designated either Ts as in Fig. 1 or T_G because of their surface receptors for IgG) react with mouse monoclonal antibodies OKT_5 and OKT_8. Their properties are discussed on p.30.

Lymphocytes exposed to antigen and mitogen release a number of factors which stimulate the monocyte/macrophage system. These "lymphokines" include a macrophage activating factor isolated from PPD. stimulated BCG. immune mouse spleen cell culture fluids (Leonard, Ruco and Meltzer, 1978). Lymphokines can enhance spreading, phagocytosis and the chemotactic attraction of macrophages (qv).

Macrophages

The monocyte/macrophage system originates in bone

marrow where monocytes mature before passing into the peripheral circulation. Some of them later become tissue macrophages.

It is uncertain whether the monocyte/macrophage system responds directly to tumour antigen. Macrophages carry Fc receptors so that tumour cells coated with immunoglobulin can be expected to bind directly to them (and possibly to produce activation) (Kjeldsberg and Pay, 1978). However indirect stimulation certainly does occur. This is mainly effected by the release of lymphokines from stimulated lymphocytes. These lymphocytes are the probable source of monocyte chemotactic factor which imparts directional mobility to monocytes and of macrophage activating factor to which reference has already been made. Monocyte chemotaxis has been measured in lung cancer patients and found to be normal except in advanced cases (McVie, Logan and Kay, 1977). Macrophages may be activated directly under experimental conditions by injection of a variety of bacterial antigens including BCG. and C. parvum.

Evidence that macrophages kill tumour cells comes from animal experiments. For example macrophages have been shown to be cytotoxic to mouse lymphoma cells in culture (Evans and Alexander, 1972). Certain macrophage activators such as Brucella abortus ether-extract (Schultz, Pavlidis and Chirigos, 1978) reduced the development of pulmonary metastases in mice in whom tumour cells had been transplanted or injected intravenously. In

contrast, macrophage poisons such as silica and carageenan increased the growth of pulmonary metastases in mice transplanted with an ovarian carcinoma (Mantovani, Giavazzi and Polentarutti, 1980). In humans Müller and Kolb (1979) found a marked infiltration of macrophages within the septae and alveoli of patients with lung cancer. Finally, adherent cells, principally macrophages, isolated from lung tumours were found to be cytotoxic to autologous tumour cells in 17 out of 25 cases by Vose (1978).

REASONS FOR FAILURE OF IMMUNOLOGICAL DEFENCE MECHANISM

With such a complex and varied defence against tumour cells, why do tumours ever develop? Possible answers can be considered under four headings:

1. Failure of recognition
2. Serum blocking factors
3. Suppressor cells
4. Intrinsic lymphocyte defects

It has been suggested that tumour cells are only weakly antigenic and are not therefore recognised as foreign. Evidence to the contrary has already been cited for lung cancer from which numerous tumour-specific antigens have been isolated. Coating of tumour cells by antibody and/or immune complexes might also mask their foreign nature. The fact that injected tumour cells are rendered more immunogenic by neuraminidase which removes coating sialic acid residues suggests that such coating might also screen tumour cells from the immunological surveillance mechanism *in vivo*.

Reference has already been made to the blocking factors in serum in connection with the inhibitory action of serum from cancer bearing animals and with immune complexes (page 15). Serum from 67/81 cancer patients blocked the cytotoxic effect of their lymphocytes on autologous tumour cells and allogeneic tumour cells of the same histological type (Hellström, Sjögren, Warner and Hellström, 1971). Another form of inhibitor present in serum of cancer patients is monocyte chemotactic factor inactivator. This has been described in 90% of patients with lung and prostatic cancer using a chemotactic agent made from E.coli. In 45% of these monocyte chemotaxis was defective. In 2/4 lung cancer patients undergoing surgical resection of tumour, this factor disappeared after operation (Kjeldsberg and Pay, 1978). In contrast, depressed Fc receptor activity of pulmonary alveolar macrophages has been attributed to local release of a blocking factor by tumours (Rhodes, Plowman, Bishop and Lipscomb, 1981).

Suppressor Cells

Suppressor cells inhibit cells or activities concerned with the immunological defence mechanism. There are two main types:

(a) Adherent cells which are removed by passage through Sephadex G 10 columns and which are believed to belong to the monocyte/macrophage series.

(b) Non-adherent cells, a subpopulation of T lymphocytes,

designated T_s cells whose properties have been extensively reviewed by Gershon (1980). These T_s cells are characterised by certain L_y surface antigens. There are different subsets of T_s cells with varying abilities and properties. Gershon and his colleagues postulated a suppressor cell circuit with inducer, amplifier/precursor, amplifier and effector cells. T_s cells suppress helper T cells, B cells and macrophages and form part of a negative feedback system which regulates the immunological defence mechanism.

Suppressor cells were detected in 11/20 lung cancer patients by Jerrells, Dean, Richardson and Herberman (1979). In 7/11 the suppressor cells were monocytes and 5 of these 7 had received BCG. immunotherapy. T_s cells are believed to make up a significant proportion of the lymphocytes which infiltrate lung tumour tissue (Vose and Moore, 1979).

Lymphocyte Deficiency

This project and a review of many investigations of immunological function in lung cancer will show that there are some abnormalities of cell-mediated immunity in established lung cancer. Whether these are merely the result of the existence of tumour cells or the cause of the development of these cells is not known. Jerrells, Dean and Herberman (1978) postulated that the abnormal lymphocyte reactivity found in lung cancer patients with normal counts of total lymphocytes and T cells may be due to depletion of a specific subpopulation of T cells.

This group of T cells formed rosettes at 29°C as well as at 4°C and were thus known as high affinity E-rosette forming cells. It could thus be that depletion of certain subpopulations of lymphocytes may play a part in the development of lung cancer.

CHAPTER 3

THE DEVELOPMENT OF IMMUNOTHERAPY IN LUNG CANCER

(a) Use of tumour cells or extracts

Attempts to stimulate host defences against tumours were first made at the end of the last century. The impetus for these seems to have come from the concept of immunity to infection and the development of active immunisation against diseases such as smallpox. Despite the enormous gulf between immunisation against future infection and immunisation against established cancer, some workers embarked on a series of immunological experiments on animals and on humans with advanced cancer. The animal experiments had an obvious defect in that tumours under investigation were transplanted into wild, randomly selected animals and not into the carefully selected inbred species used in modern laboratories. Hence tumour rejection, which at the time seemed promising, may have been due to simple allograft tissue rejection rather than specific antitumour resistance (Currie, 1972).

Many of the early investigations in humans involved a form of specific active immunotherapy. Von Leyden and Blumenthal (1902) injected 2 patients with advanced cancers of urethra and uterus and ovaries with filtrates of their own tumour tissue and observed some reduction in size of enlarged lymph nodes though no change in the overall progress of their disease. During the next 50 years sporadic similar investigations were carried out. Assessment of the clinical effect of such treatment was largely subjective and in few studies were prospective controls used. In a few, some attempt was made to measure

the immunological results of treatment. For example, Vaughan (1914) observed that anticancer antiserum raised in animals was clinically effective only when its use was followed by an increase in circulating mononuclear cells.

The majority of early attempts at specific immunotherapy in humans were carried out on patients with advanced tumours especially those involving breast and gastrointestinal tract. As the first successful resection of lung cancer did not occur until 1933 (Graham and Singer, 1933) bronchial carcinoma never featured in earlier studies of immunotherapy where autologous tumour tissue was used. While a few cancer patients seem to have derived some benefit from treatment (Vaughan, 1914) it was ineffective in the majority. Side effects were fortunately rare. In particular, local growth of tumour at the site of injection of tumour cells was rare although only Kellock, Chambers and Russ (1912) irradiated the cells before injection. Enhancement of growth of the primary tumour or metastases was also an infrequent occurrence (Risley, 1911).

Between 1950 and 1960, workers began to measure the immunological effect of immunotherapy. Graham and Graham (1955) prepared antigens from gynaecological tumours removed at operation. They used these to demonstrate antibodies to tumour antigen which were present in 12/48 patients. The same workers found a three fold rise in serum gamma globulin in 6 of 35 patients treated with

autologous tumour vaccines (Graham and Graham, 1959). Twenty-six out of 60 patients had a significant increase in epithelial cells with densely staining finely vacuolated cytoplasm in vaginal secretions. Their presence indicated that the tumour was more likely to respond to radiotherapy. Finney, Byers and Wilson (1960) measured serum antibody levels in 9 patients treated with autologous tumour vaccine in complete Freund's adjuvant, one of whom had a lung cancer. The antibody levels rose to a plateau 30 days after injection and remained at the same level for the remaining 60 days of the study.

Some of the early animal trials of specific immunotherapy were carried out at the Chester Beatty Research Institute. For example, Haddow and Alexander (1964) injected irradiated autologous tumour cells into rats bearing benzpyrene-induced fibrosarcomas. This increased tumour response to radiotherapy.

No adjuvant was used in most early studies of specific active immunotherapy in cancer although Southam (1960) commented on the illogicality of removing cancer tissue from a patient and injecting it elsewhere in the same patient unless it was rendered more immunogenic by purification, concentration or addition of adjuvant.

Thus by 1970, the use of adjuvant in trials of specific immunotherapy had become established. In the much quoted investigations of Mathé, BCG. was found to enhance the effect of irradiated autologous leukaemia cells in delaying growth of L. 1210 leukaemia grafted into mice

(Mathé, Pouillart and Lapeyraque et al., 1969a) and a similar form of immunotherapy was used with apparent success in humans with acute lymphoblastic leukaemia (Mathé, Amiel, Schwarzenberg et al., 1969b).

(b) Bacterial antigens

The use of bacterial antigens to stimulate host resistance to tumours started around the turn of the century. Loeffler (1901) described injection of staphylococci and later tubercle bacilli in patients with advanced cancer. Use of tubercle bacilli was suggested by the observation that tuberculosis and cancer rarely occurred in the same patient. McCaskey (1902) found that less than 2% of 281 patients dying with cancer had autopsy evidence of tuberculosis. Pearl (1929) found active tuberculosis in 6.6% of 816 patients with malignant growths compared with 16.3% of patients free of cancer. These observations were in agreement with the epidemiological discovery that the fall in incidence of tuberculosis between 1891 and 1911 was paralleled by a rise in incidence of cancer (Cherry, 1924).

After the second world war, there was renewed interest in bacterial antigens as anticancer agents. BCG. vaccination had been introduced for the prevention of tuberculosis in 1921 (Calmette and Guérin, 1924). In 1959, Halpern, Biozzi, Stiffel et al. described retardation of the growth of injected tumour in rats by intravenous BCG. and the Grahams reported on the use of complete Freund's adjuvant (Myobacterium hominis in mineral oil suspension) in patients with advanced cancers, (Graham and Graham, 1959).

Corynebacterium parvum, an anaerobic organism, was found by the French group to stimulate the reticulo-endothelial system of mice (Halpern, Prévot, Biozzi et al., 1964). Injection of the same organism into these animals 2 days before and between 8 and 12 days after subcutaneous inoculation of viable mammary carcinoma delayed the growth of the implanted tumour (Woodruff and Boak, 1966). Thus mycobacterial antigens and C. parvum were available as single agents or as adjuvants at the start of the great immunological attack on lung cancer which started after 1965.

(c) Immunotherapy in lung cancer

Methods of immunotherapy used in lung cancer are summarised in Figure 2 (from Stack, 1980). Most of the early work involved the use of non-specific methods. These consisted of introducing agents which cause a general stimulation of all cells concerned with the immunological response in the hope that some of the activated immuno-competent cells would attack the tumour cells. Bacterial antigens have been much the most popular stimulants of non-specific immunity and the majority of these have been mycobacteria or extracts of these organisms.

Most of the early trials of non-specific immunotherapy in lung cancer were carried out in patients with advanced disease. For example, Hadziev and Kavaklieva-Dimitrova (1969) treated 71 patients with stages 3 and 4 lung cancer with intradermal BCG. and found increased mean

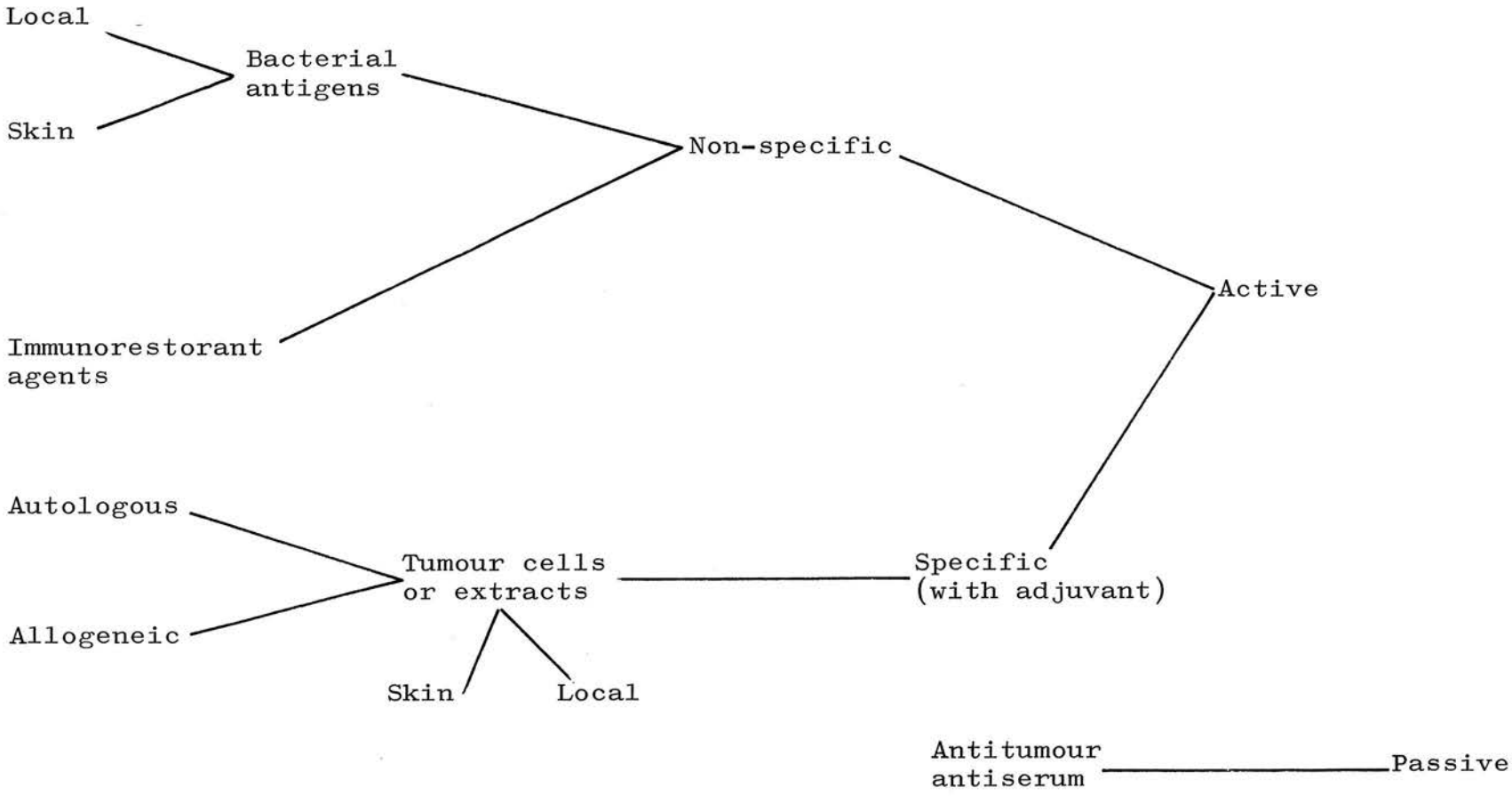

Figure 2. Methods of immunotherapy used in lung cancer.

survival compared with controls. Israel (1974) found that combination of Corynebacteria (parvum or granulosum) with chemotherapy produced better survival of inoperable cases than chemotherapy alone.

In one of the earliest prospective trials in operable cases, Edwards and Whitwell (1974) found initial promising results from post-operative subdermal BCG. Only a single, relatively low dose was given and this early promise was not confirmed in subsequent reports (Edwards and Whitwell, 1978).

Immunorestorants are drugs or extracts which restore depressed cell-mediated immunity to normal. They might thus be expected to be of value during the post-operative period when immunological function is depressed (see later). An early report of the use of levamisole suggested that it might be a valuable adjuvant to surgery in operable cases (Study group for bronchogenic carcinoma, 1975). However these results have since been confirmed only for patients in whom the dose of levamisole exceeded 2.1 mg/Kg (Amery, 1980). Use of this drug was associated with an excess death rate from cardiorespiratory failure in one series (Anthony, Mearns, Mason et al., 1979).

Passive immunisation has not been widely employed. Newman, Ford, Davies and O'Neill (1977) gave intravenous anti-serum raised against tumour cells in goats to some patients receiving chemotherapy after resection of bronchial carcinoma. Although there were more deaths and tumour recurrence among the controls, the difference was not significant.

Specific immunotherapy involving the injection of lung tumour cells or extracts was also tried on inoperable cases initially. Takita and Brugarolas (1973) used autologous tumour cells, antigenic protein and complete Freund's adjuvant, and Tallberg (1974) administered polymer particles coated with autologous tumour extract. The early results suggested that such treatment might be beneficial and it was on this basis that the West of Scotland Lung Cancer Group embarked on the present study in 1975.

CHAPTER 4

PILOT TRIAL

Patients

Preliminary details of this trial were given by Stack, McSwan, Stirling et al. (1979). Informed consent for the investigation was obtained from patients undergoing thoracotomy for suspected bronchial carcinoma at the Regional Cardiothoracic Centre, Mearnskirk Hospital, between November 1st 1975 and May 1st 1976. In order to obtain randomly selected comparable groups, a series of cards were prepared which were divided between male and female and between the age groups less than 40, 40-49, and 50-69. At the time of operation, once the surgeon had decided that the tumour was resectable, a telephone call was made to the clerical assistant who then randomly selected an envelope containing one of the cards. This allocated the patient to the autograft or non-autograft group.

Preparation and injection of tumour cell suspension

The excised tumour was transected and a 0.5 cm cube of solid tumours macroscopically free from fat and necrosis, was excised and placed on a 100-mesh stainless steel gauze (Anderson, Kelly, Wood et al., 1973). The tissue was diced into small pieces with scalpels and then ground down using a glass pestle. The material was then washed through the gauze using 20 mls of Eagle's medium (a balanced salt solution containing penicillin, streptomycin and antimycotic agents). The resulting suspension, which was about 20 mls in volume, was placed in a sterile plain glass container. No further antibiotics and no enzymes were added. This container was transferred

immediately to the Radiotherapy Department of Belvidere Hospital for irradiation.

Irradiation of the tumour cell suspension was achieved by using a special applicator which allowed the cell containers to be placed as near as possible to the source of radiation. A minimum dose of 12,250 rads x-ray therapy (half value layer 2.5 mm.cu.) was delivered to the cells in a single dose. Occasionally super voltage irradiation was employed, a dose of 14,280 rads being delivered to obtain the same biological effect.

After irradiation the tumour cell suspension was divided between 5 or 6 tubes, each tube containing 1.5 mls of suspension.

Provided that the diagnosis of lung cancer had been confirmed by a frozen section taken at the time of operation, two tubes containing 3 mls of tumour cell suspension were returned to the Cardiothoracic Unit and the remainder was despatched to the laboratory where it was quenched in liquid nitrogen at $-210^{o}C$. A small proportion however was injected onto two blood agar plates in order to detect contamination with aerobic organisms and into two bottles of Robertson's meat medium in order to detect anaerobic infection. No contamination occurred in the pilot trial.

The cell suspension that was returned to the Cardiothoracic Unit was mixed with 0.5 mls of standard intradermal BCG. Glaxo ($8 - 26 \times 10^{6}$ viable units per ml). This cell suspension mixed with BCG. was then injected in four different sites intradermally and subcutaneously

into the anterior and lateral aspects of the thighs. This injection of autograft was repeated two and ten weeks after operation (Figure 3).

Radiotherapy

At the time of operation a braided steel marker was placed in the stump of the upper lobe bronchus. Three weeks after operation, or occasionally a little later depending on the patient's condition, a course of radiotherapy was given to the mediastinum in the region of the hilum as indicated by the steel marker. The full course of radiotherapy consisted of 3,500 rads tumour dose which was given in 16 treatments over 22 days.

Immunological Tests

Immunological screening of the patient was performed two weeks before operation, at 2, 7 and 12 weeks after operation and at three monthly intervals thereafter over the course of two years. During this time, none of the patients were taking corticosteroids or immunosuppressive drugs.

Skin Tests

Tuberculin Test

0.1 ml of Tuberculin PPD., Weybridge (10 Old Tuberculin units) was injected intradermally into the volar surface of the forearm. The maximum diameter of induration at 48 hours was recorded.

DNCB. Test

Different strengths of dinitrochlorobenzene (DNCB.) were made up by dissolving DNCB. crystals in acetone. The patient was sensitised to DNCB. by placing 3 drops of 2%

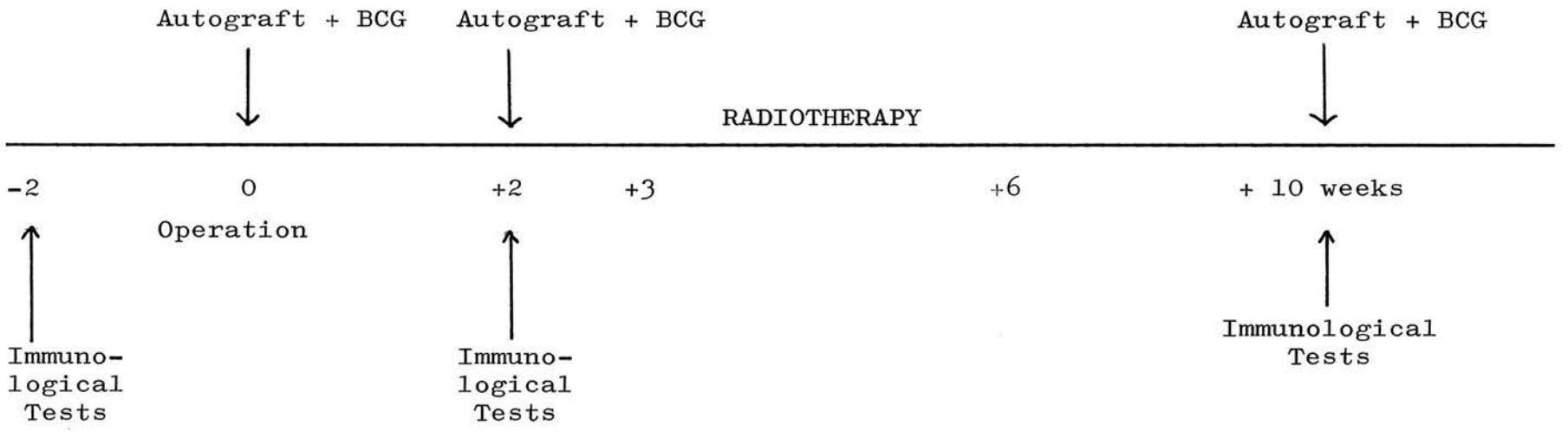

Figure 3. Scheme of pilot trial.

DNCB. on the skin inside a 2 cm diameter brass ring which was fastened to the skin by micropore. The DNCB. was dried onto the skin after evaporation. The patient was instructed not to wash the arm for 48 hours.

Between 10 and 14 days later a strip of 5 patches was applied to the volar surface of the other forearm (Figure 4).

These patches were impregnated with DNCB. in the following dilution: 62.5, 125, 250, 500 and 1000 micrograms per ml. The strip was fastened to the volar surface of the forearm and removed 2 days later. The reaction was graded from 1 to 4 according to the degree of erythema and oedema produced at each site. A positive DNCB. reaction, analogous to a positive tuberculin test, was taken as erythema covering the area of the disc treated with 500 micrograms per ml. (Figure 5).

Laboratory Tests of Immunological Function

At each immunological screening, 35 mls of venous blood was withdrawn. 5 mls were placed in a sequestrene tube and sent to the haematology laboratory for estimation of the total and differential white cell count from which the total lymphocyte count was derived.

30 mls of blood was divided between 3 heparinised tubes and transferred as quickly as possible to the laboratory. At the same time similar blood samples were taken from controls who were on the same hospital premises at the same time. These controls were either healthy members of staff over the age of 40 or other patients

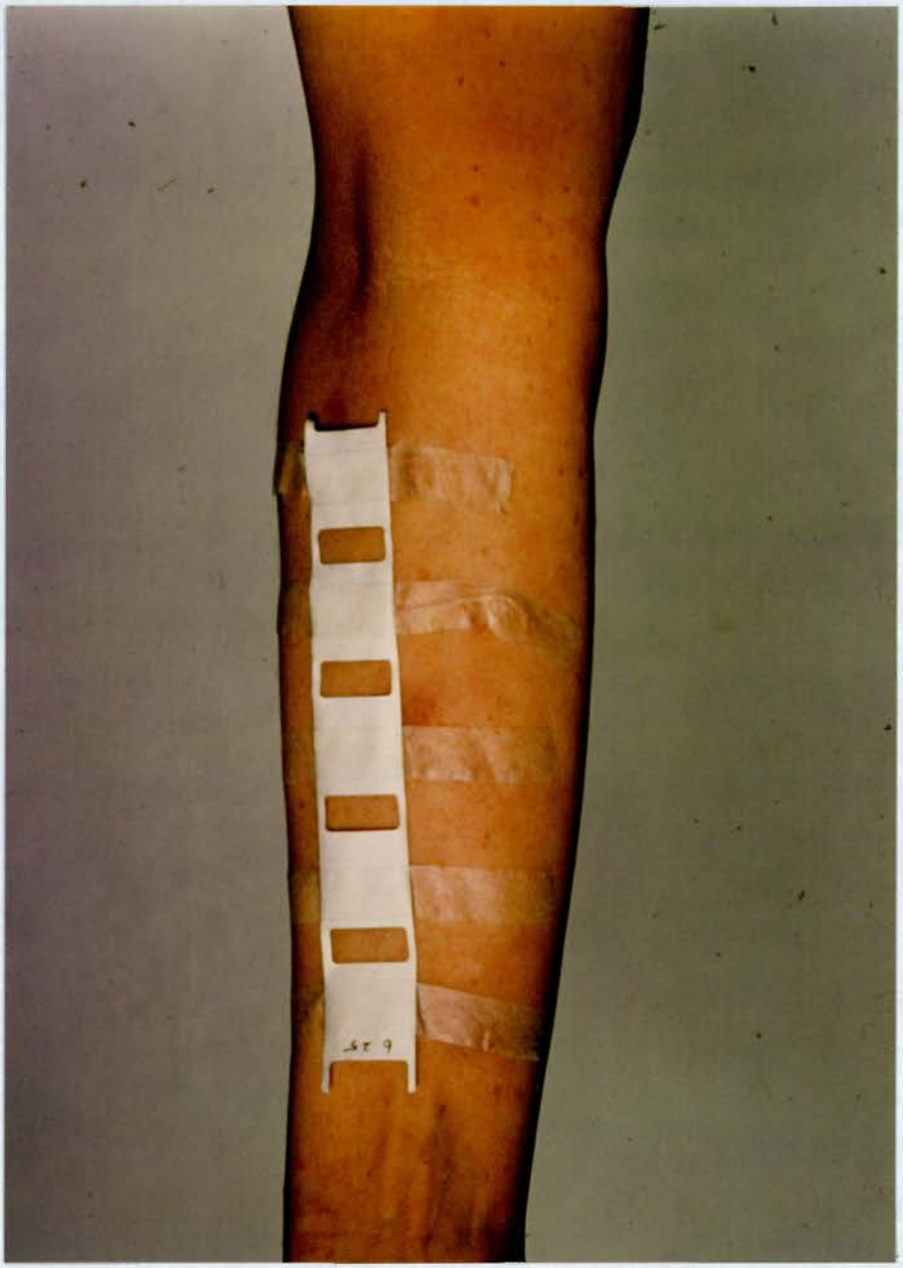

Fig. 4 D.N.C.B. impregnated patches in strip applied to forearm.

Fig. 5 Strongly positive reactions to D.N.C.B. at 48 hours.

who were not suffering from malignant disease and who were not taking immunosuppressive drugs.

Estimation of T and B cell counts

Twenty mls of heparinised whole blood was separated on Ficoll-Triosil mixture to provide lymphocytes for the estimation of T and B cells by the rosetting technique and for the lymphocyte transformation studies (MacKie, Sless, Cochran and de Sousa, 1976). For T lymphocyte count, 0.25 ml aliquots of lymphocyte suspension were incubated for 15 - 20 mins. at 37°C with an equal volume of 1% washed sheep red blood cells. The suspension was centrifuged at a low speed with the formation of cell pellets. These were incubated for 24 hours at 4°C. The cells were then gently re-suspended and the percentage of rosette-forming cells was estimated. A rosette-forming cell was defined as a lymphocyte whose surface was touched by a minimum of 3 red blood cells. 200 cells were counted in all.

For the estimation of B lymphocytes, 0.25 ml aliquots of a 1% suspension of sheep red blood cells which had been freshly coated with 1/2000 rabbit anti-sheep red blood cell haemolysin and normal human plasma as a source of complement were centrifuged at low speed with an equal volume of the lymphocyte suspension to form cell pellets. These pellets were incubated together at 37°C for 15-20 minutes and then re-suspended. The number of rosette-forming lymphocytes was then counted. The count by this method will have included residual monocytes in the

lymphocyte-rich preparations but such contamination has been shown to be < 5% of total cells recovered after Ficoll-Triosil separation.

Lymphocyte Transformation Studies

0.5×10^6 lymphocytes were incubated in culture medium in a moist 37°C chamber with 5% CO_2. The culture medium used was Eagle's medium buffered with 2% Hepes buffer and enriched with 10% foetal calf serum. Phytohaemagglutinin (PHA., Wellcome) was used at a final dilution of 1 in 10 and pokeweed mitogen (PWM.) (Grand Island Biological Company) was used at a dilution of 1 in 5. The cultures were incubated for 72 hours. ^{14}C-thymidine was added for the last 4 hours (0.05 microcuries per tube). After extraction of cell suspensions with 10% trichloracetic acid and 2 ml absolute alcohol, the filter paper was dried in air for > 4 hours. 5 ml. of liquid scintillator was then added. The radio-activity was measured in a Packard tricarb scintillation counter and expressed as counts per minute per 10^6 cells. Transformation ratios were calculated as ratios of the uptake of ^{14}C-thymidine after exposure to the mitogen to uptake when no mitogen was added to the culture medium.

Out-patient Follow-up

Patients were seen at the out-patient department for the first time 10 weeks after operation and 4 weeks after the course of radiotherapy. Thereafter they attended at three monthly intervals until two years after operation and at six monthly intervals up to five years after

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operation. At each attendance, they were seen by Dr. I. McHattie and the author. A history of relevant complaints was taken and a physical examination to detect evidence of tumour recurrence or metastases was performed. The patient had a postero-anterior chest radiograph. Scans of bone, brain or liver were carried out as indicated. Finally a note was made about the patient's activity and employment. This information was recorded on a standard form (Figure 6).

Statistical Methods

Student's t test was used to determine significant differences in all the immunological tests except for those of lymphocyte transformation by mitogens and PPD.. In the latter, a Wilcoxon 2 sample test was used, because of the lack of normal distribution of the readings. Statistical analysis was performed by Mr David J. Hole, Statistician, The Cancer Surveillance Unit, Ruchill Hospital.

Histology and Staging

The histology of the tumour tissue removed at operation was determined with reference to the WHO histological classification of lung tumours. TNM. staging was performed by a pathologist from a different hospital (Dr. W.G.S. Spilg, Victoria Infirmary) who was unaware of the treatment given and the subsequent progress of any of the patients. The tumour of each patient was allocated to one of three stages as indicated by the American Joint Committee for Cancer Staging and End-results Reporting, (1979).

Survey No. Radiotherapy No.

Assessment Weight Change

Clinical evidence of recurrence	Present Yes	Present No	Change	Radiographic evidence of recurrence	Present Yes	Present No	Change
Chest pain				Mediastinal broadening			
Haemoptysis				Pulmonary shadow			
Dyspnoea				Pericardial effusion			
Dysphagia				Diaphragmatic involvement			
Persistent hoarseness				Other			
SVC obstruction							
Other							
Cervical nodes				Brain scan			
Distant nodes				Bone scan			
Brain				Opposite lung			
Liver				Opposite pleura			
Bone							
Opposite lung							
Opposite pleura							
Skin							

√ = present, + = increase, — = decrease.

Radiotherapy since last visit

...ed	Date completed	Dose	Site

Antimitotic drugs

...ed	Date completed	Drug	Dose

Immunotherapy Yes/No

Dose (ml).....

Reaction .....

.....

D.N.C.B. .....

Tuberculin .....

...6 Form completed at each formal assessment of the patients.

PILOT TRIAL RESULTS

PRE-OPERATIVE MEASUREMENTS

The Patients

The basic clinical details of the 15 patients in the Pilot Trial are given in Table 1. The age, sex and mean measurements of body size were similar in the two groups. All except one of the patients had been smoking cigarettes up to the time of hospital admission. One patient had given up smoking three years previously.

Pathology

Pathological details of the tumours are given in Table 2. It can be seen that there was a higher proportion of squamous cell carcinoma in the non-autograft group. In two cases the co-existence of substantial areas of squamous cell carcinoma and adenocarcinoma tissue led the pathologist to include both histological diagnoses. There was a higher proportion of Stage III carcinomas in the non-autograft group.

Operation and Radiotherapy

A higher proportion of the autograft patients underwent lobectomy. Only five patients in each group completed post-operative radiotherapy. The reasons for failure to complete treatment were: early post-operative death (1), patient refusal to continue with treatment (1), poor general condition (1), and poor condition combined with painful leg ulcers (2).

	AUTOGRAFT	NON-AUTOGRAFT
Total	8	7
No. of females	1	2
No. of smokers	7	7
Mean Age	58	53
Mean Weight (kg)	72.5	66.3
Mean Height (cm)	174	169
Mean $FEV_{1.0}$ (% of predicted)	78	69

Table 1. Pilot Trial patients: initial clinical details.

		AUTOGRAFT	NON-AUTOGRAFT
Histology	Squamous cell carcinoma	2	5
	Large cell carcinoma	3	-
	Adenocarcinoma	1	2
	Squamous cell/ adenocarcinoma	2	-
Stage	I	6	4
	II	1	0
	III	1	3
Operation	R. side operation	3	3
	Lobectomy	6	4
	Pneumonectomy	2	3
Radio-therapy	Completed course	5	5
	Total in each group	8	7

Table 2. Pathology, staging and treatment in Pilot Trial.

Immunological Profile

In order to show that randomisation produced two groups of patients with comparable immunological function I have drawn up Table 3 which gives the mean pre-operative results in the two groups. DNCB. testing was not carried out pre-operatively in the pilot trial.

POST-OPERATIVE CLINICAL RESULTS

Clinical Measurements

A considerable degree of caution is necessary in drawing conclusions from the clinical results of the pilot trial because of the small numbers involved.

1. Survival Data

A survival curve is shown in Figure 7. Table 4 gives the percentage of patients surviving at different intervals up to five years after operation. The overall results are similar. Although the autograft group appear to have fared worse in that only two out of eight patients survive at the end of five years, two of the six deaths in this group were due to causes other than tumour recurrence whereas in the control group three of the four deaths were due to tumour recurrence. The number of deaths due to tumour recurrence were thus 4 and 3 respectively in the two groups.

2. Freedom from Tumour Recurrence

Figure 8 and Table 5 show the proportion of patients in each group alive and free from clinical and radiographic evidence of tumour recurrence during the five years after operation. It can be seen that there is no significant difference between the two groups.

	AUTOGRAFT (8)	NON-AUTOGRAFT (7)
Total wbc x 10^9/l	8.9	10.4
Total lymphocytes/ cu.mm.	2392	2731
% T cells	47	40
T cell number/cu.mm.	1079	1254
% B cells	27	31
B cell number/cu.mm.	741	1005
PHA. ratio	185	115
Pokeweed ratio	52	41
Tuberculin test (mm)	23	19

Table 3. Mean values of immunological measurements in autograft and non-autograft patients in pilot trial.

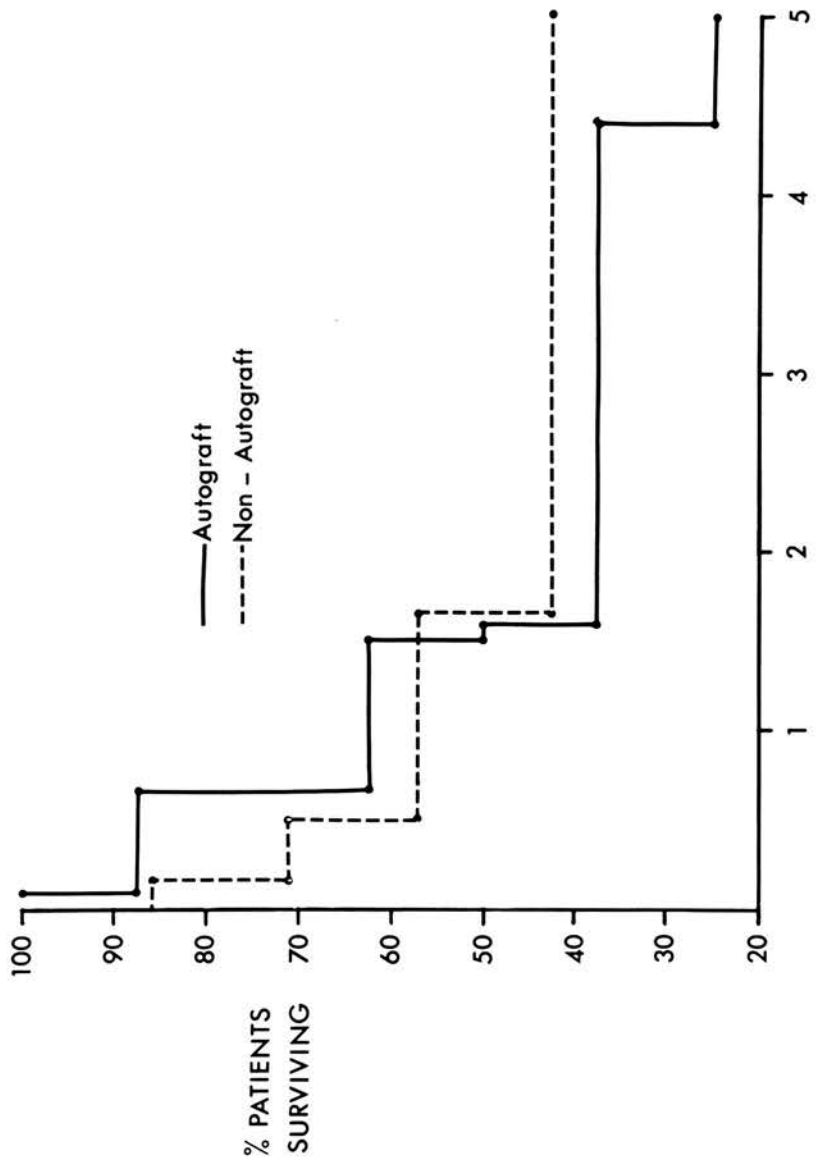


Fig. 7 Percentage of patients in pilot trial surviving after operation.

	1 year		2 years		3 years		4 years		5 years	
	No.	%	No.	%	No.	%	No.	%	No.	%
Autograft Group	5	62.5	3	37.5	3	37.5	3	37.5	2	25
Non-autograft Group	4	57.2	3	42.9	3	42.9	3	42.9	3	42.9

Table 4. Proportion of patients surviving at each year after operation in pilot trial.

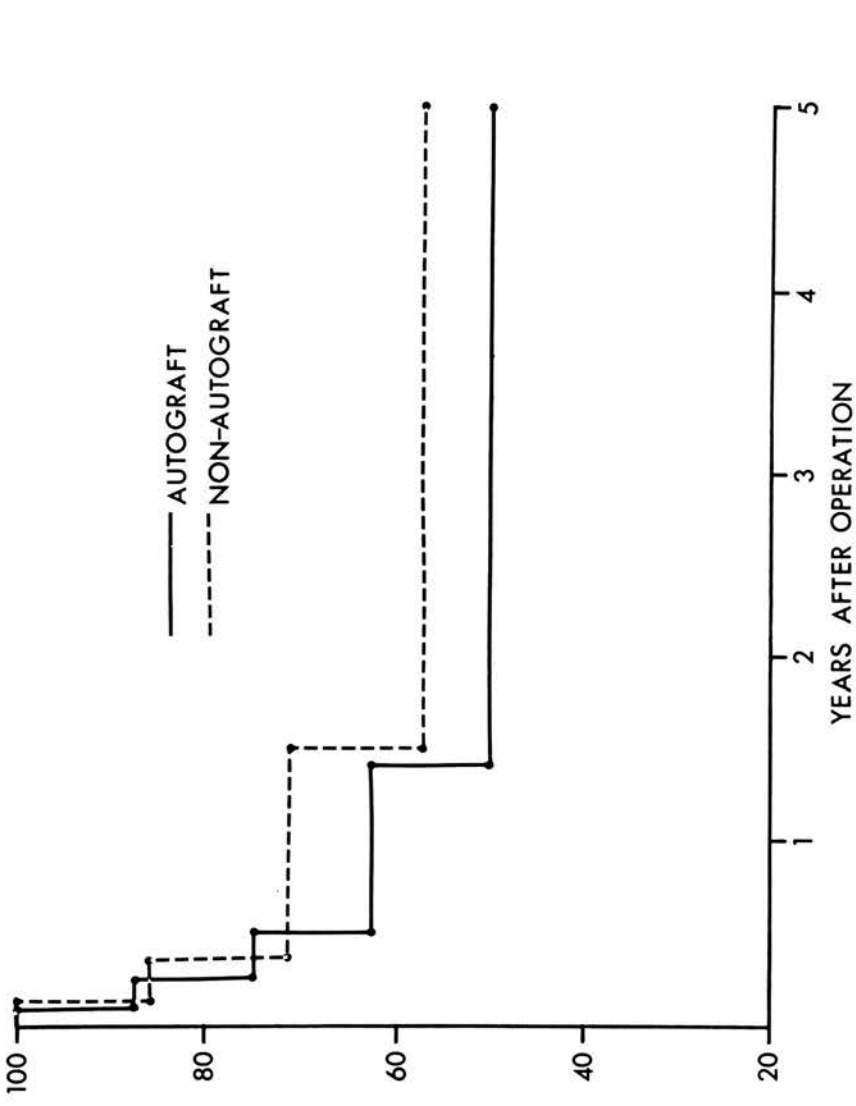


Fig. 8 Percentage of patients in pilot trial alive and free from tumour recurrence after operation.

% Free of Recurrence	1 year		2 years		5 years	
	No.	%	No.	%	No.	%
Autograft group (8)	5	62.5	4	50	4	50
Non-autograft group (7)	5	71.5	4	57.2	4	57.2

Table 5. Freedom from clinical evidence of tumour recurrence of patients in the pilot trial.

3. The sites at which metastases first appear (Table 6).

There is no support for the view that systemic immunotherapy reduces the incidence of distal metastases relative to local recurrence.

4. Activity

In Table 7, an attempt has been made to assess the quality of life of those patients who were alive one year after operation. Again no difference was noted between the two groups.

5. Unwanted effects

Five of the seven patients treated with intradermal irradiated autologous cells and BCG. developed significant fever during the first 48 hours after treatment. By the end of 48 hours some induration and erythema at the site of injection could be seen and this increased during the next few weeks. By two months after operation, fluctuant swellings were present at the injection site and during the next two months these discharged cellular and fluid debris with the formation of ulcers. In one case these were between 10 and 25 cms in diameter (Figure 9). The ulcers persisted from six to nine months and then started to heal. By one year healing with scar tissue formation had taken place (Figure 10).

All five patients with severe local reactions had tuberculin test results in excess of 20 mm. In contrast the two patients with relatively mild reactions had initial tuberculin tests at or below this value.

No patients developed clinical or biochemical evidence

Site of tumour recurrence	Autograft (4)	Non-autograft (3)
Local	1	1
Liver	1	1
Brain	2	1
Skin	1	0

Table 6. Site of initial tumour recurrence in the pilot trial patients.

Grade of Activity	Autograft (5)	Non-autograft (4)
Full activity	3	3
Limited activity	2	1
In hospital	0	0

Table 7. Level of activity one year after operation in the pilot trial.

Fig. 9 Ulcers on anterior surface of thigh of pilot trial patient six months after injection of B.C.G. and autologous tumour cells.

Fig. 10 Healed lesions on anterior surface of thigh of pilot trial patient one year after injection of B.C.G. and autologous tumour cells.

of liver function impairment and in the two patients who died from tumour recurrence and had autopsies, granulomatous hepatitis was not reported.

Ten patients completed post-operative radiotherapy and seven of these had lobectomy. A striking feature was the degree of fibrosis and shrinkage which occurred in the remaining lobe on the operated side during the following year. Figure 11 shows the chest radiograph taken one year after left lower lobectomy in one patient from the autograft group who had a full course of radiotherapy. In contrast Figure 12 shows a chest radiograph taken one year after right lower lobectomy in a patient from the main trial who did not receive post-operative radiotherapy. The pilot trial patient and one other patient from the same group subsequently developed infection with Aspergillus fumigatus involving the fibrotic lung.

Post-operative immunological results

It should be appreciated that early in this study, laboratory measurements were not achieved on all the specimens submitted for immunological tests. The reason for this included the clotting of cells, especially where the cell count was high, insufficient numbers of cells in the specimen, or failure of cells to resuspend after centrifugation. This fact, coupled with the death of three patients during the first six months, meant that the number of results recorded at any one time more than three months after operation was insufficient for analysis.

Fig.11. Chest radiograph taken one year after left lower lobectomy in a pilot trial patient who had a full course of radiotherapy. It shows extensive opacification and shrinkage of the remaining lobe of left lung.

Fig. 12. Chest radiograph taken one year after right lower lobectomy in a main trial patient who did not receive radiotherapy.

Laboratory Measurements

A major problem with laboratory measurement of immunological function is the wide variation in results recorded in the same patient over a period of time. Hence in the pilot trial, where the number of patients was small, changes in the mean value, which appeared striking when displayed graphically, were not always significant when analysed statistically. In all the figures shown, the post-operative results are given as a change from the mean pre-operative results and significant differences ($p < 0.05$) are indicated by an asterisk. These results can be summarised as follows:

1. Total leucocyte count (Figure 13)

   A rise two weeks after operation followed by a fall during the period following radiotherapy in the autograft group but no change in the non-autograft group.

2. Total lymphocytes

   A fall in total lymphocyte count, significant only in the autograft group (Figure 14) after radiotherapy.

3. T lymphocytes (percentage, Figure 15, and absolute numbers, Figure 16). A rise in the autograft group at seven weeks followed by a significant fall in both groups presumably resulting from radiotherapy.

   There was no significant change in the percentage and absolute numbers of B cells, or in reactivity to PHA. or PWM.

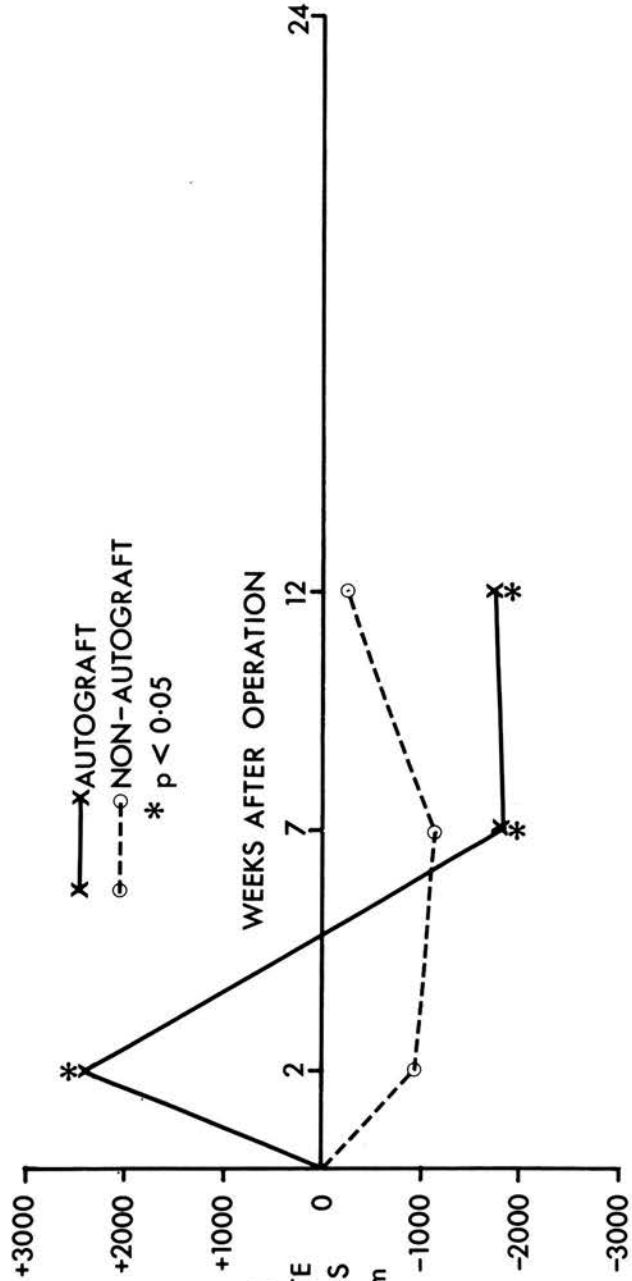


Fig. 13 Mean change in total w.b.c. count during postoperative period in pilot trial.

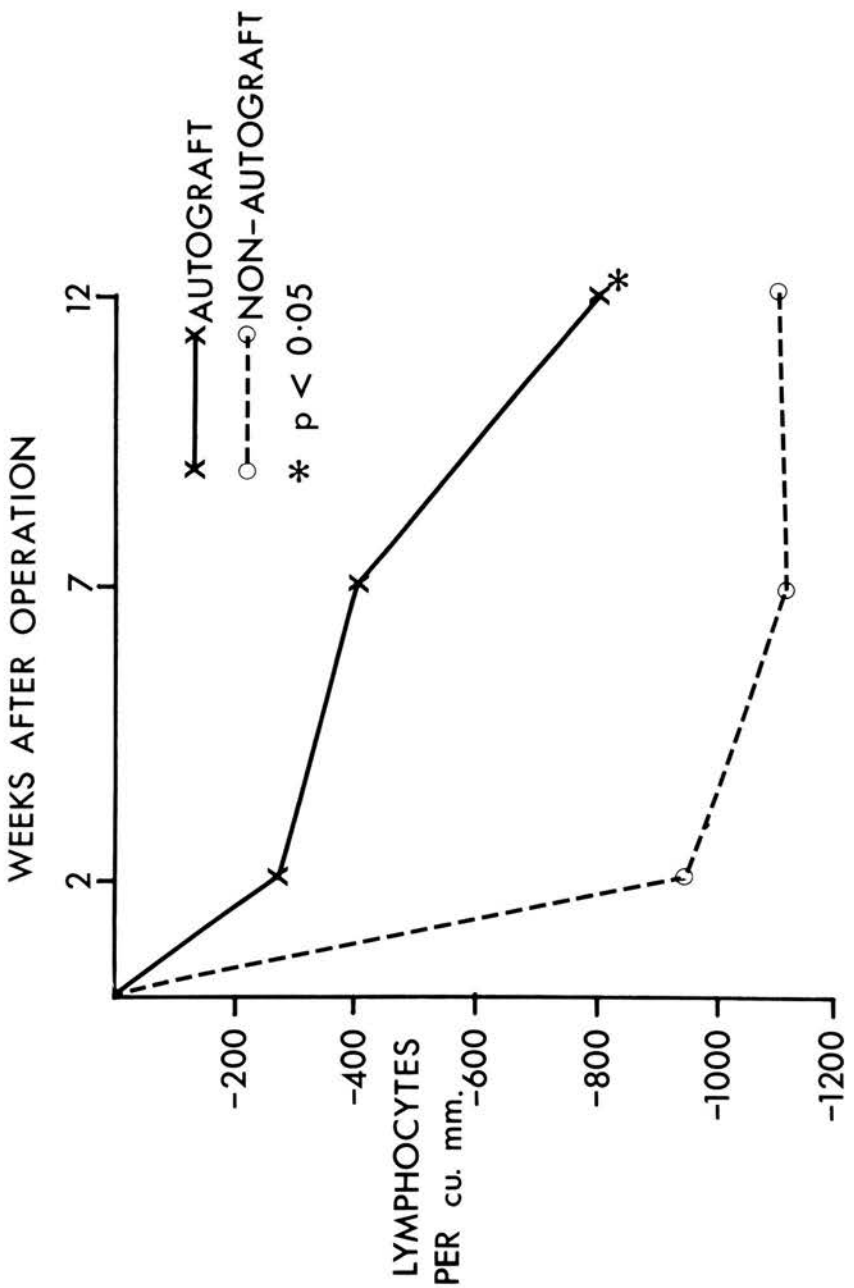


Fig. 14 Mean change in total lymphocytes after operation in pilot trial patients.

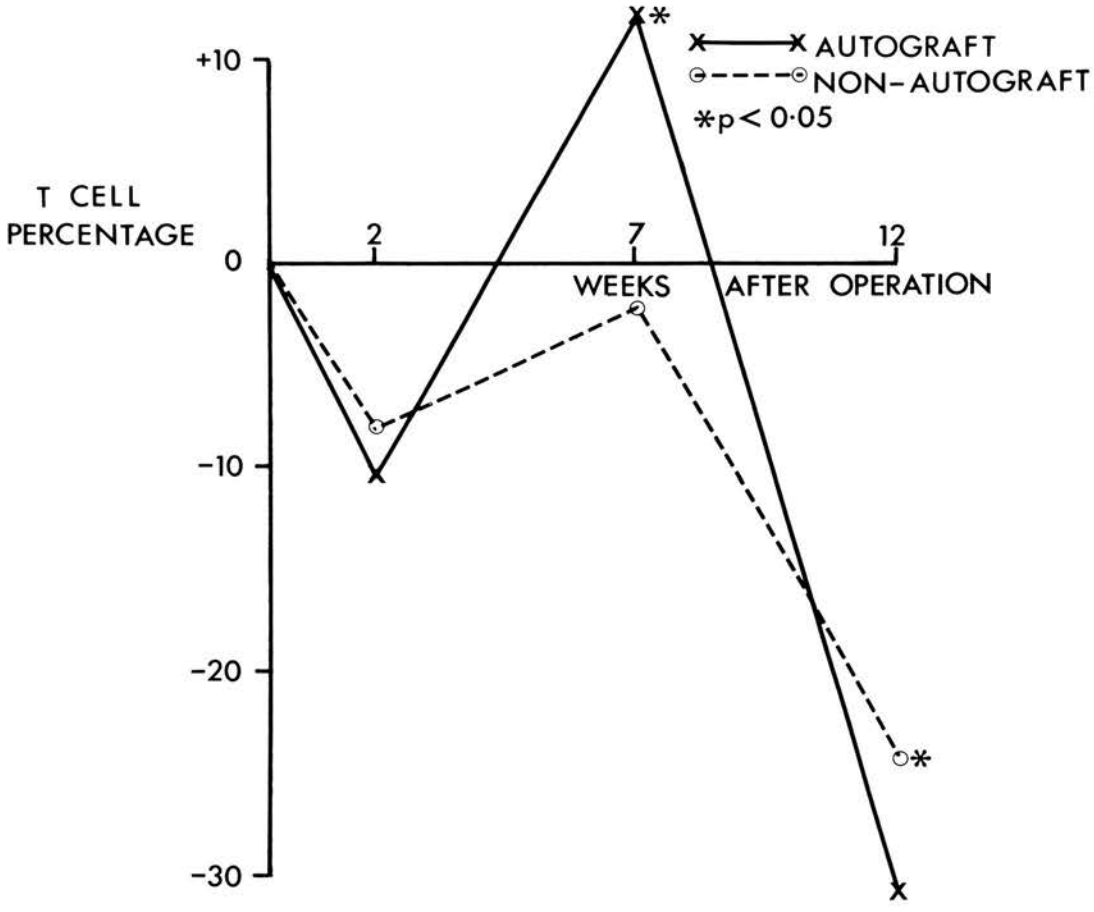


Fig. 15 Mean change in percentage T cells after operation in pilot trial patients.

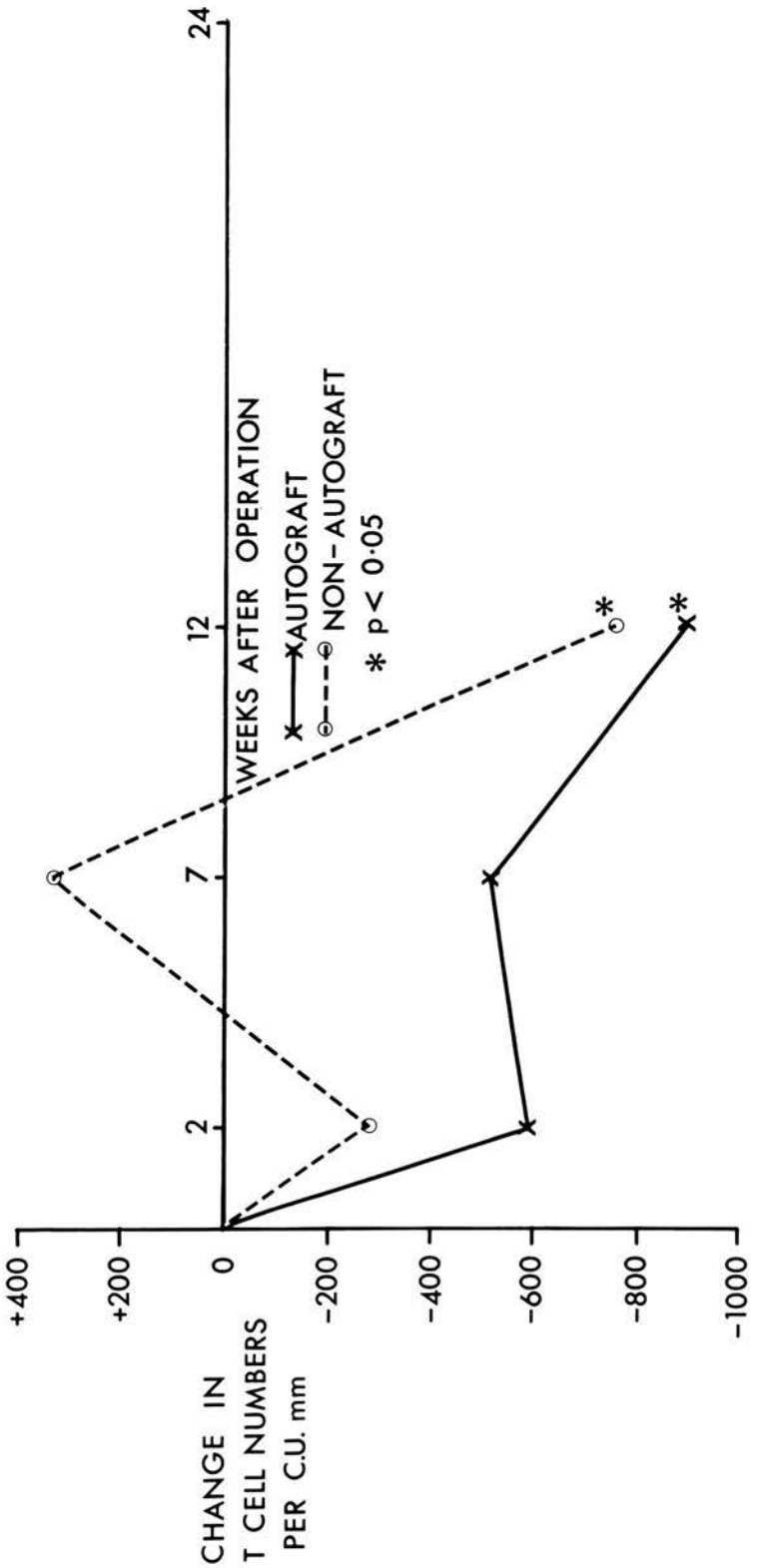


Fig. 16 Mean change in absolute numbers of T cells after operation in pilot trial patients.

Tuberculin Test

Tuberculin reactivity increased at two weeks in the autograft group and subsequently decreased in both groups after radiotherapy (Figure 17). DNCB. reactivity was only tested post-operatively in the pilot trial. It increased equally in both groups on the second challenge, a finding which was repeated in the main trial.

MODIFICATION OF THE PILOT TRIAL PROTOCOL

Our preliminary results suggested that this form of specific immunotherapy had produced changes in immunological function which might be beneficial to the patient. Unfortunately two elements in the treatment protocol were together producing unacceptable discomfort to the patients.

The lesions on the legs were painful and made walking difficult. This discomfort was becoming marked at the time at which the patients underwent their course of radiotherapy. This involved transport by ambulance or taxi to another hospital some 15 miles away. The combined effects of the leg lesions and the daily travel caused the withdrawal of three patients from radiotherapy and it was followed by a strong plea from our surgical colleagues for a change in the protocol.

By that time other workers had been using a multiple puncture method of administering BCG. percutaneously and this had produced considerably less local reaction than that which our patients had experienced (Horne, 1976; Pines, 1976). Because the local reaction was most severe in patients with a strong tuberculin reactivity, it seemed

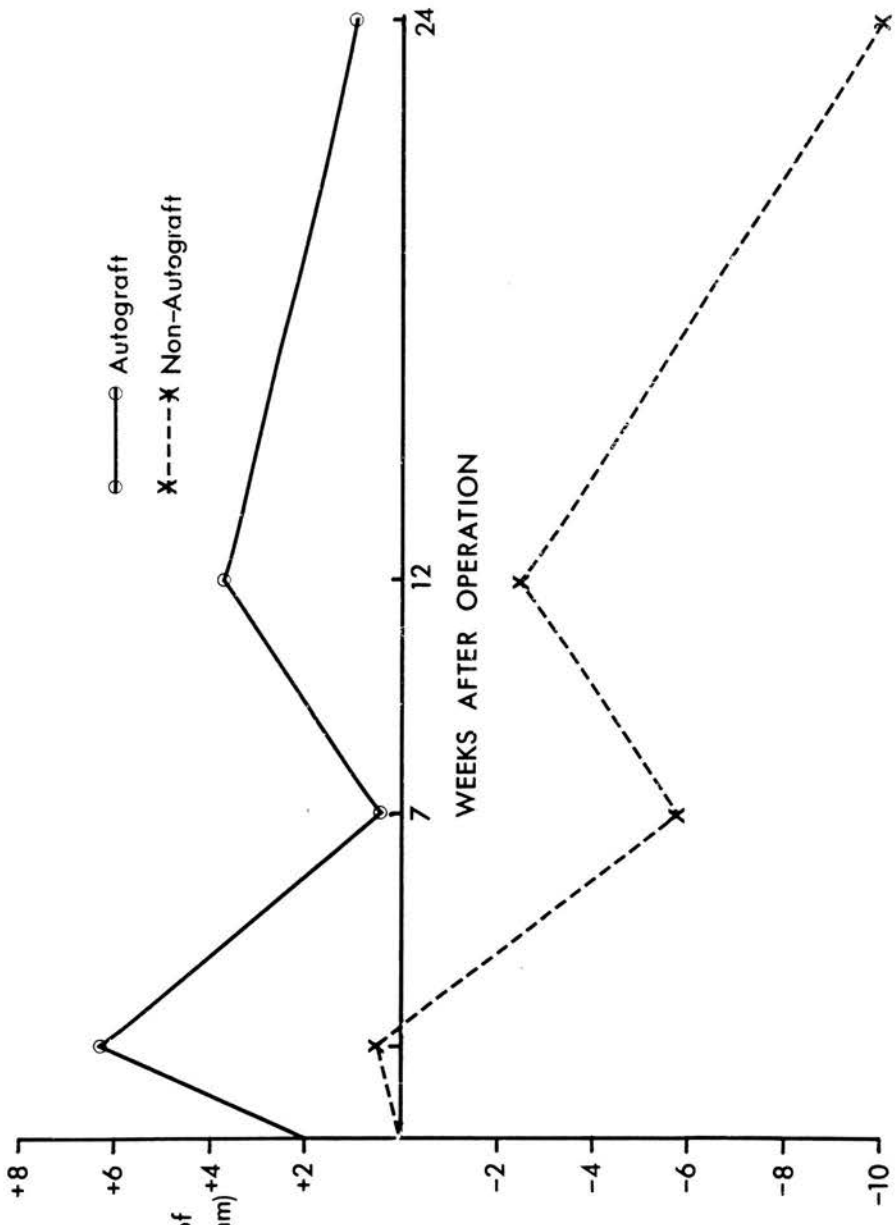


Fig. 17 Mean change in maximum diameter of induration at 48 hrs. of tuberculin reaction after operation in pilot trial patients.

likely that intradermal BCG. rather than the injection of cells was mainly responsible for the ulceration. We therefore decided to administer BCG. by this percutaneous technique using a 20 needle Heaf gun. Moreover, because the thighs were thought to be more prone to infection, the deltoid region of the upper limbs was chosen for the BCG. injection.

Radiotherapy had been given because of the belief that an important role of immunotherapy was to render the tumour more sensitive to radiotherapy. This belief was based on the effect of irradiated autologous tumour cells in increasing the effectiveness of radiotherapy in delaying the rate of growth of benzypyrene-induced sarcomas in rats (Haddow and Alexander, 1964). However previous investigations of post-operative radiotherapy in patients undergoing resection of bronchial carcinoma had not shown striking improvement (Paterson and Russell, 1962). For this reason it was decided that radiotherapy would be omitted from the protocol in the future.

By that time, Powles et al.(1973) had been injecting allogeneic irradiated tumour cells into limbs treated previously with percutaneous BCG. This technique led to the idea that BCG. might be used to "prepare" the lymph nodes adjacent to the site of injection for a response to injection of autologous irradiated cells. Because it was felt that the first injection of these cells should be given immediately after operation when the cells were fresh, it was necessary to give one

percutaneous treatment with BCG. to all patients before operation. The "Main Trial" can therefore be considered as a comparison of strong repeated post-operative specific immunotherapy with weak single dose pre-operative immunotherapy.

CHAPTER 5

THE MAIN TRIAL

Methods

The scheme of the main trial, entered by the first patient on August 23rd, 1976 and the last patient on August 23rd, 1979 is given in Figure 18. The procedure was generally similar to that of the pilot trial and the changes can be considered under the following headings:

Immunological tests.

Immunotherapy regime.

Omission of postoperative radiotherapy.

Follow-up attendance.

Immunological tests

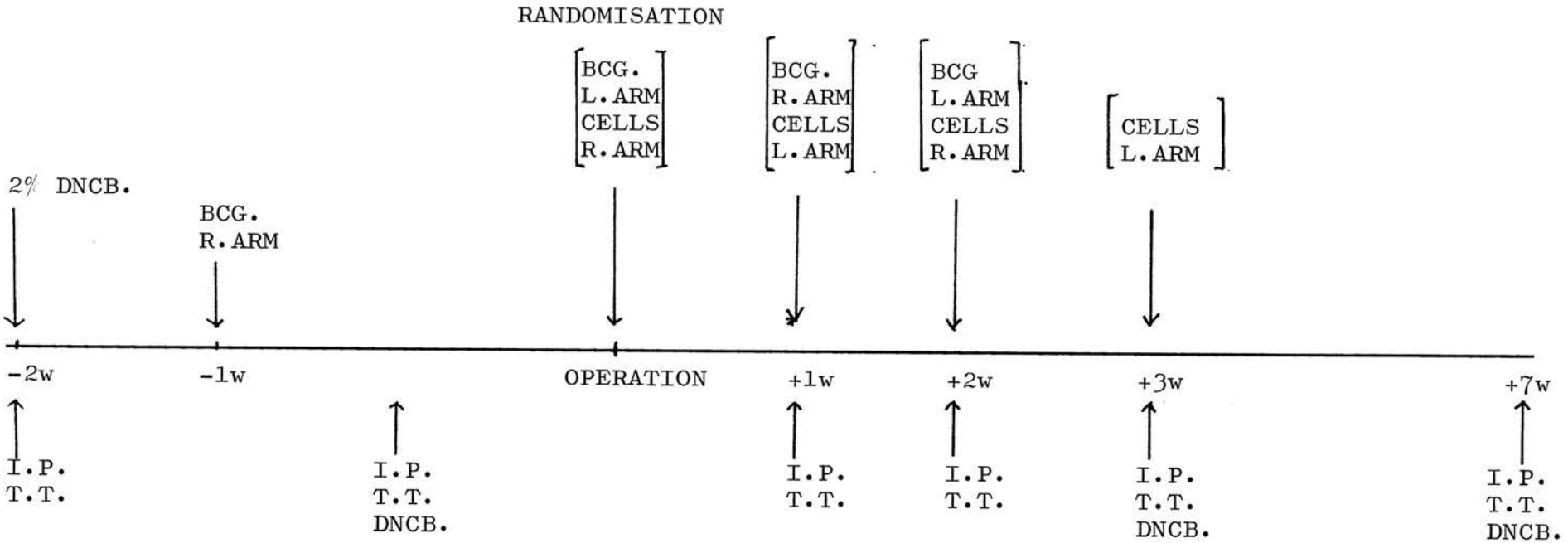
(a) Routine

Ten to 14 days before operation for suspected bronchial carcinoma, a tuberculin test and laboratory tests of immunological function were performed. At the same time, a sensitising dose of 2% DNCB. was applied to the forearm. Between 2 and 4 days before operation these tests were repeated and, in addition, challenge strips containing weak dilutions of DNCB. were applied to the other forearm. These were read 48 hours later, just before operation. The tuberculin and laboratory tests were repeated at 1, 2, 3 and 7 weeks and the DNCB. at 3 and 7 weeks after operation.

(b) Laboratory methods

Lymphocyte transformation by PPD.

The same laboratory tests were used as those in the pilot trial except that lymphocyte transformation by PPD.



I.P. - IMMUNOLOGICAL PROFILE

T.T. - TUBERCULIN TEST

TREATMENT DENOTED BY [ ] REFERS TO THAT WHICH IS GIVEN TO AUTOGRAFT GROUP ONLY.

Figure 18. Scheme of Main Trial.

was measured. 100,000 units/ml. PPD. were dialysed against distilled water to remove phenol (preservative) and sterilised by millipore filtration to remove contaminants. This was diluted so that 50 µl. added to 1 ml. of culture medium gave concentrations of 100 units/ml. to which the lymphocytes were exposed. Transformation ratios were calculated as ratios of uptake of ^{14}C-thymidine after exposure to PPD. to the uptake when no mitogen was added, as for the mitogen-induced lymphocyte transformation tests described in the pilot trial.

Immunotherapy regime

(a) Routine

Patients who were believed to have resectable lung cancer received percutaneous BCG. by 20-needle Heaf gun into the deltoid region of one arm approximately one week before operation. Randomisation and, in those allocated to the autograft group, preparation of the cell suspension were carried out as in the pilot trial.

On the day of operation, patients in the autograft group were injected intradermally and subcutaneously with 3 ml. of tumour cell suspension. The injection site was the deltoid region of the arm. The range of cell counts was 0.3 to 68.0 x 10^6/ml. and the mean cell count was 25.1 x 10^6/ml. At the same time percutaneous BCG. was injected into the deltoid region of the other arm. During the next 3 weeks the autograft patients received serial injections of tumour cells and percutaneous BCG. into the deltoid regions of both arms so that the cells were injected

into an arm "primed" with BCG. the previous week.

(b) Percutaneous BCG. vaccination

0.3 ml. sterile water was added to freeze-dried powdered BCG. Glaxo and the mixture agitated until complete mixing had taken place with the production of a suspension containing 50 to 250 x 10^6 viable organisms per ml. This was drawn up into a syringe and dropped over the injection sites. A heat-sterilised 20-needle Heaf gun was adjusted to give an injection depth of 1 mm. and applied to 5 sites in the region covered with BCG. suspension. At each site, two sets of injections were made, by rotating the gun through 90° for the second injection. Thus 5 injection sites with 40 needle punctures each were produced (Figure 19).

Omission of Radiotherapy

No post-operative radiotherapy was given in the main trial.

Follow-up Attendance

Patients were usually seen at the hospitals one month after discharge, approximately 7 weeks after operation, 12 weeks after operation and thereafter at 3 monthly intervals up to 2 years. After 2 years they were seen at 6 monthly intervals. Follow-up of patients in both trials continues at the present time. Tuberculin tests were performed at each follow-up attendance during the first 2 years after operation. The majority of these and the second post-operative DNCB. test were read at 48 to 54 hours in the patients' homes by the author but a

Fig. 19 Deltoid region of a female patient in the main trial 3 weeks after percutaneous injection of B.C.G. using a multiple puncture Heaf gun.

few attended Mearnskirk Hospital for reading of the tests by Mr N. McSwan.

Measurement of DNCB. reactivity

In order to obtain a measure of DNCB reactivity, dose response curves were constructed for each patient according to their reaction to increasing strengths of DNCB.. The DNCB. index was read off from the curve as the strength of reaction (between 0 and 4) at a concentration of 250 μg/ml.

Until September 30th, 1979, laboratory tests of immunological function were performed at each attendance during the first 2 years after operation. With the cessation of the Cancer Research Campaign grant, these tests were no longer available. Hence in patients entering the main trial in 1978 and 1979, some of the postoperative laboratory immunological data are incomplete although all these patients had regular skin tests.

Extension to the Western Infirmary

In the main trial, 11 patients operated on by Mr M.A. Turner at the Western Infirmary were recruited.

Comparison of autograft and non-autograft groups

Clinical, surgical, pathological and immunological data from both groups of patient are shown in Table 8. There is an excess of lobectomies among the control group and of large cell carcinomas among the autograft group, both of which might be expected to operate to the disadvantage of the autograft group. Reference will be made to the different proportions of T_1N_0 patients among stage I patients between the two groups. (p.158)

		Autograft Patients (N = 40)	Non-Autograft Patients (N = 43)
Age (yrs)	Mean	56.5	57.8
	Range	45-68	47-69
Sex	Males	30	34
	Females	10	9
Operation	Lobectomy	21	31
	Pneumonectomy	19	12
Histology	Squamous cell	27	33
	Large cell anaplastic	7	1
	Adenocarcinoma	4	7
	Mixed + other	2	2
Stage at	I	24	30
	(T_1N_0	13	9)
	2	1	1
	3	15	12
Mean tuberculin reaction (mm)		15.8	17.9
Mean DNCB. reactivity (units)		1.4	1.2

Table 8. Characteristics of operable bronchial carcinoma patients in main trial.

CHAPTER 6

IMMUNOLOGICAL MEASUREMENTS BEFORE OPERATION

RESULTS

Combined pilot and main trial

No significant difference was found in the counts of total white blood cells (wbc) and total lymphocytes and in the percentages of T and B cells between patients and controls (Table 9). The percentages of B cells measured were rather higher than would be expected with the methods in use today but this did not affect the comparison of the two groups. From Figure 20 it can be seen that, whereas lymphocyte reactivity to PHA. and PWM. were similar in patients and controls, lymphocyte reactivity to PPD. was significantly depressed in the lung cancer patients ($p < 0.001$). In Figure 21 it can be seen that the percentage of patients and controls with a positive tuberculin test was similar but that a much smaller proportion of patients compared with controls became sensitised to DNCB.. This latter test was performed before operation only in patients from the main trial.

MAIN TRIAL RESULTS

Immunological measurements before operation in relation to the tumour and the clinical outcome

In Table 10, the preoperative immunological tests in patients with stage 1 and stage 3 tumours are compared for the main trial. Total lymphocytes ($p = 0.08$), total B cells ($p = 0.06$) and the percentage of DNCB. positive patients ($p = 0.06$) were appreciably lower in patients with more advanced tumours. Tuberculin reactivity was greater in patients with large cell anaplastic carcinoma and adenocarcinoma than in squamous cell carcinoma ($p < 0.05$)

	Patients	Controls	
Total w.b.c.	9216	8535	per cu.mm.
Total lymphocytes	2663	2480	per cu.mm.
Percentage T cells	46	48	%
Percentage B cells	31	27	%

Table 9. Mean counts of total leukocytes and lymphocytes with mean T and B cell percentages in combined pilot and main trial patients.

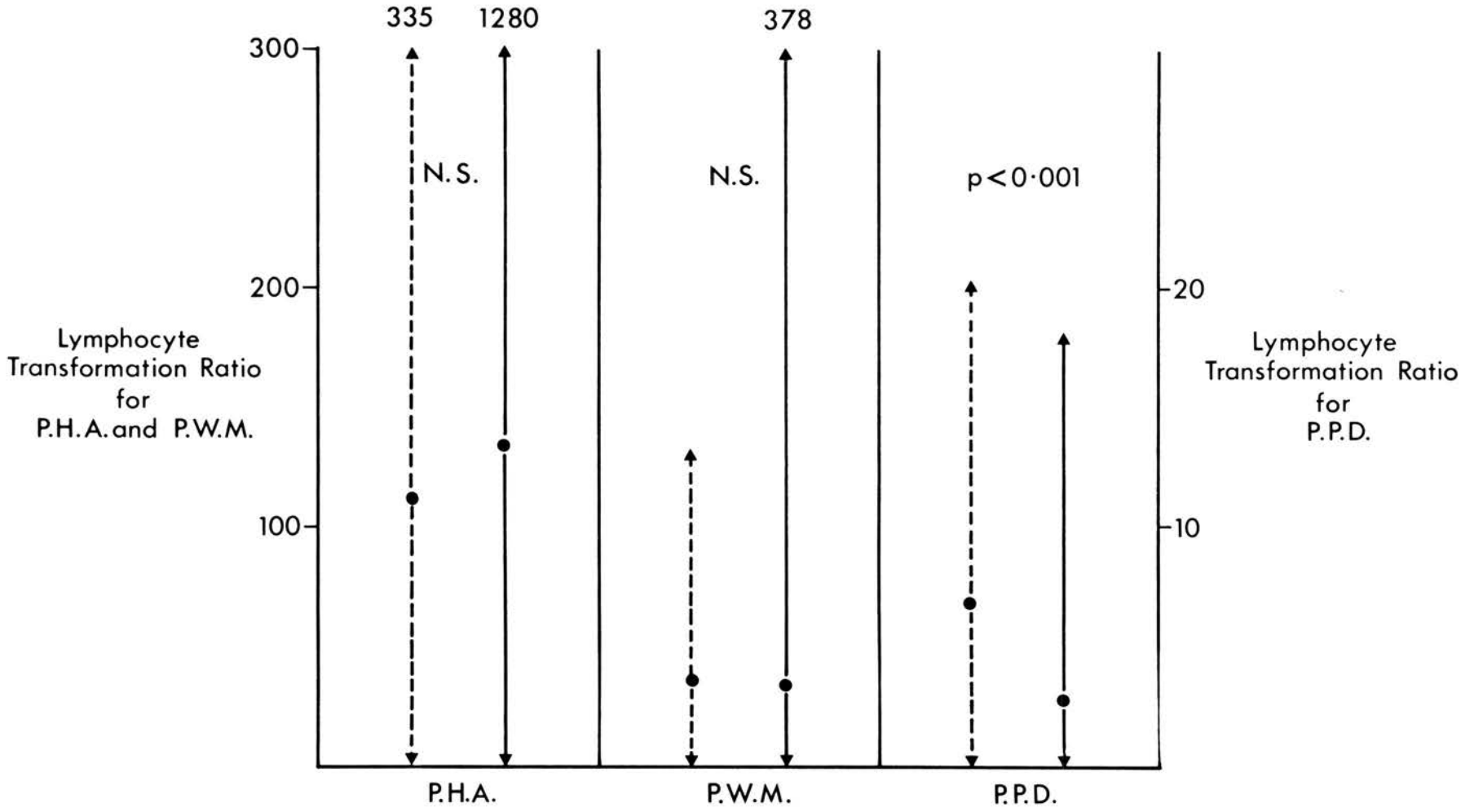


Fig. 20 Median lymphocyte transformation ratios with P.H.A., P.W.M. and P.P.D. in lung cancer patients before operation (solid line) and controls (interrupted line) showing depressed lymphocyte reactivity to P.P.D. in lung cancer

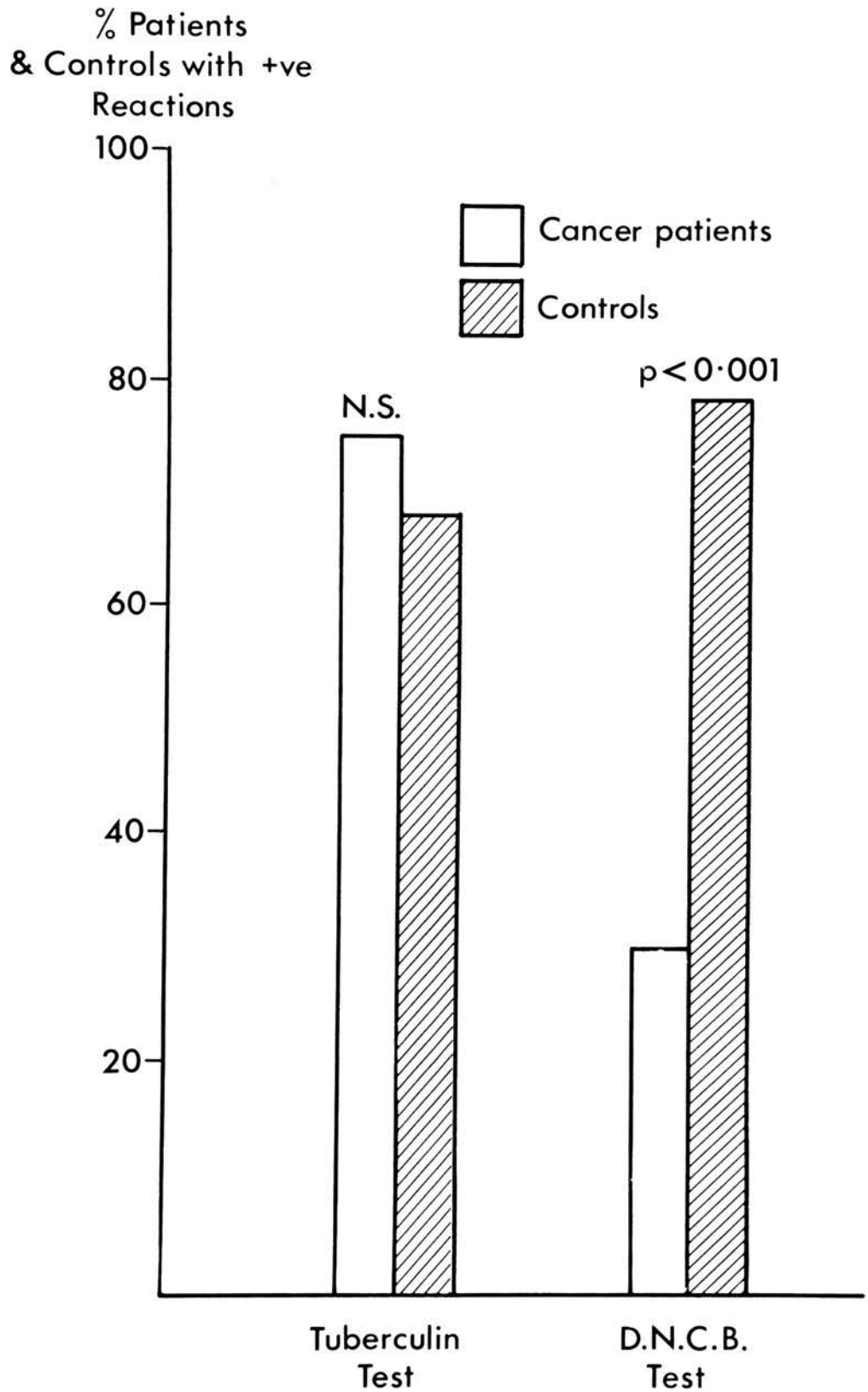


Fig. 21 Percentage of lung cancer patients before operation and controls who had positive reactions to tuberculin and D.N.C.B.

	Stage 1	Stage 3	
Total w.b.c.	9243	8235	per cu.mm.
Total lymphocytes	2666	1898	per cu.mm.
Total T cells	1063	578	per cu.mm.
Percentage T cells	38	33	%
Total B cells	898	335	per cu.mm.
Percentage B cells	28	23	%
Lymphocyte transformation ratios			
PHA.	126	163	
PWM	29	43	
PPD	2.2	2.6	
Delayed hypersensitivity skin tests			
Tuberculin test	19.5	14.0	mm.
Percentage positive DNCB. test	44	18	%

Table 10. Immunological results from combined pilot and main trial patients showing reduced total lymphocytes, B cells and DNCB. reactivity in Stage 3 compared with Stage 1 patients.

but otherwise there was no significant difference between different histological types (Table 11).

In Table 12 the immunological results in patients surviving more than 2 years after operation are compared with those of patients who died within this period. Patients surviving more than 2 years had a higher mean total lymphocyte count ($p = 0.02$). Otherwise there was no significant difference between the groups. When patients surviving 2 years without tumour recurrence and those who had no evidence of recurrence at the time of death were compared with patients who developed tumour recurrence within 2 years of the operation, the total lymphocyte count ($p = 0.08$) and the incidence of DNCB. sensitisation ($p = 0.05$) were found to be reduced in the tumour recurrence group (Table 13).

Correlation of lymphocyte transformation test results with these different pathological and clinical features proved disappointing. This was undoubtedly due to the wide variability of results within groups and within patients over a period of time. Cell counts and delayed hypersensitivity skin (DHS.) reactivity were more stable and less variable. These measurements seemed more reliable in assessing immunological status.

DISCUSSION

Peripheral blood cell counts

The finding of similar levels of total wbc., total lymphocytes, and T and B cells in patients and controls is in agreement with the results of Ritts *et al*. (1977)

	Squamous cell	Large cell anaplastic	Adeno-carcinoma	Mixed cell type	
Total w.b.c.	9108	8457	7118	7933	per cu.mm.
Total lymphocytes	2508	2007	2107	1920	per cu.mm.
Total T cells	1018	700	644	574	per cu.mm.
Percentage T cells	36	31	37	36	%
Total B cells	741	278	524	375	per cu.mm.
Percentage B cells	25	15	26	17	%
Lymphocyte transformation ratios					
PHA.	129	161	178	50	
PWM.	35	35	30	8	
PPD.	2.1	1.0	3.2	1.5	
Delayed hypersensitivity skin tests					
Tuberculin test	15	22	23	9	mm.
Percentage positive DNCB. test	36	0	25	17	%

Table 11. Mean values for immunological tests and percentage positive DNCB. reactors in main trial patients with tumours of different histologies. Significant difference ($p < 0.05$) between combined adenocarcinoma and large cell carcinoma groups and squamous cell carcinoma group in mean tuberculin reaction.

	Death from lung cancer < 2 years	Alive at 2 years	
Total w.b.c.	8529	8913	per cu.mm.
Total lymphocytes	2093	2698	per cu.mm.
Total T cells	770	1075	per cu.mm.
Percentage T cells	36	36	%
Total B cells	721	559	per cu.mm.
Percentage B cells	27	23	%
Lymphocyte trans-formation tests			
PHA.	106	164	
PWM.	27	38	
PPD.	2.0	2.2	
Delayed hyper-sensitivity skin tests			
Tuberculin test	14.6	18.5	mm.
Percentage positive DNCB. test	23	39 .	%

Table 12. Mean pre-operation values for immunological tests and percentage positive DNCB. reactors in main trial patients in relation to survival during first 2 years after operation. Total lymphocytes are significantly higher in those surviving more than 2 years ($p = 0.02$).

	Recurrent disease within 2 years	Disease free at 2 years or time of death	
Total W.b.c.	8543	8938	per cu.mm.
Total lymphocytes	2186	2638	per cu.mm.
Total T cells	802	1065	per cu.mm.
Percentage T cells	36	35	%
Total B cells	722	539	per cu.mm.
Percentage B cells	26	22	%
Lymphocyte transformation tests			
PHA.	113	161	
PWM.	28	38	
PPD.	2.1	2.1	
Delayed hypersensitivity skin tests			
Tuberculin test	15	18	mm.
Percentage positive DNCB. test	21	43	%

Table 13. Mean pre-operation values for immunological tests and percentage positive DNCB. reactors in main trial patients in relation to recurrence of lung cancer within 2 years of operation. Total lymphocytes ($p = 0.08$) and DNCB. reactivity ($p = 0.06$) higher in tumour free group.

and Roberts, Donohoe, Hewitt and Evans (1977) for localised lung cancer (Table 14). In contrast, two groups cited found depressed T cells even in these early cases and depression of total and T cells has been found in unselected series of patients. In general, both total lymphocytes and T cells become depressed in more advanced cases. B cell elevation has been reported in squamous cell carcinoma with regional extension (Ritts et al, 1977) but in no other series.

This considerable discrepancy in findings may well be due to the use of different control groups. Evidence of the effect of smoking on immunological function and the decline of this with age suggests that it is prudent to use controls matched for age, sex and smoking habits. This was done for total leukocyte and total lymphocyte counts only in this study and also by McEvoy, Cowled, McKenzie et al. (1979) alone of the authors listed in Table 14. Some workers have used as controls patients with non-malignant pulmonary disease. These will probably include a high proportion of patients with chronic bronchitis and emphsema who are cigarette smokers.

Why does lymphopenia occur in lung cancer? McMahon and Thomson (1980) have shown an inverse relationship between the lymphocyte/total wbc. ratio in peripheral blood and plasma cortisol in serial diurnal measurements. This might suggest that increased endogenous cortisol production depresses lymphocyte formation. In keeping with this theory is the finding that total lymphocyte

	Authors	Controls	Total lymphocytes	T cells
Localised	Dellon et al. 1975	Healthy, unmatched	-	↓
	Ritts et al. 1977	Chronic lung disease, minor surgical	-	=
	Roberts et al. 1977	Healthy or non-pulmonary disease	=	=
	Wybran et al. 1979	Non-malignant pulmonary disease	-	↓
Unselected	McEvoy et al. 1979	Age, sex and smoking history matched	-	=
	Prochazka et al.1980	Blood donors	-	↓
	Szczepaniec and Pieton, 1979	Non-malignant pulmonary disease	↓	↓

Table 14. Peripheral blood total lymphocyte and T cell counts in lung cancer.

= same levels of cell count
↓ cell count depressed
} relative to controls

- not done.

levels are most severely depressed in advanced cases (Roberts et al., 1977) where plasma cortisols are highest (Lichter and Sirrett, 1975).

Alternatively the relative depression of both total and T lymphocytes seen in some series (Table 14) may simply indicate redistribution of lymphocytes from peripheral blood to other sites. These sites might be tumour tissue itself where concentrations of lymphocytes have been described (Ioachim et al., 1976). This would explain the rapid return to normal of T cell levels following tumour resection (Dellon, Potvin and Chretien, 1975). Lymphocytes might also be sequestered in liver, spleen and lymph nodes. However the latter was not confirmed by Syrjänen (1979) who found that lymphocyte follicles, small and medium-sized lymphocytes were less numerous in lymph nodes draining lung cancers than in those draining gastric ulcers.

Lymphocyte mitogen induced reactivity

Lymphocyte transformation on exposure to a variety of stimulants in vitro is a commonly used non-specific test of cell-mediated immunity. The mitogens used include phytohaemagglutinin (PHA.), concanavalin A (Con A) and pokeweed mitogen (PWM.). These are known as polyclonal activators because they do not act as antigen and do not depend on prior sensitisation of the lymphocytes for their action. PHA. and Con A are thought to act mainly on T cells and PWM. on both T and B cells. PHA. has been considered the most useful mitogen for in vitro tests to distinguish between lymphocytes from lung cancer

patients and those from normal controls.

Lymphocyte transformation tests can also be performed with bacterial antigens such as PPD.. In contrast to non-specific mitogens, these act only on lymphocytes that have been sensitised to the antigen.

Numerous reports of depressed lymphocyte transformation tests in unselected series of lung cancer patients have appeared during the past 13 years of which some examples are given in Table 15. As with cell counts, more attention should have been paid to selection of controls in view of the decline of PHA. reactivity with age (Barnes et al., 1975) and the depression of cell-mediated immunity by smoking. However even where these factors were considered (McEvoy et al., 1979) PHA. reactivity was relatively depressed. It has been possible to extract data on localised lung cancer from 2 papers. There was no significant depression of lymphocyte reactivity compared with controls except for reactivity to PWM. in one report (Jansen et al., 1979a).

What is the cause of depressed lymphocyte transformation by PHA. in lung cancer? One factor may be decreased numbers of effector T cells. However this cannot be the only explanation since reduced lymphocyte transformation by PHA. has been reported in patients where the mean T cell count was normal (McEvoy et al., 1979). Another factor may be the presence of suppressor cells in the cell population studied. Passage of peripheral blood lymphocytes

Distribution	Authors	Controls	Lymphocyte reactivity to mitogen		
			PHA.	PWM.	PPD.
Localised	Jansen et al. 1979a	Chronic obstructive lung disease	=	↓	-
	Barnes et al. 1975	Healthy age and sex matched	=	-	-
Unselected	Saumon et al. 1968	Healthy, blood donors	↓	-	-
	Prochazka et al. 1979	Blood donors	↓	-	-
	Han and Takita, 1972	Healthy blood donors	↓	-	-
	Rees et al. 1975 (Stage 3)	Healthy staff	↓	-	=
	McEvoy et al. 1979	Age and sex matched controls (smokers)	↓	-	-
	Jerrells et al. 1977	Not specified	↓	↓	↓

Table 15. Reactivity to mitogens of lymphocytes from localised disease and unselected lung cancer patients.
= same level of reactivity
↓ reactivity depressed
relative to controls
- not done

from lung cancer patients through Sephadex G10 columns restored PHA. responsiveness presumably by removing adherent suppressor cells (Jerrells, Dean, Richardson et al., 1978a). Moreover recovery of PHA. responsiveness was also observed when lymphoid cells from lung cancer patients were cultured with prostaglandin synthesis inhibitors (Thomas, Huchet, Grandjon and Mathé, 1978). Suppressor cells are believed to act by production of prostaglandins. Their role in depression of cell-mediated immunity in cancer has recently been confirmed by Mavligit, Raphael, Calvo and Wong (1980) who showed that indomethacin, a prostaglandin inhibitor, restored local graft v. host reaction in cancer patients where this had previously been depressed. The fact that suppressor cells are more numerous and active in advanced cancer (Han and Takita, 1978) would explain why depression of PHA. activity was not seen in the operable cases in this series and in the 2 reports of localised disease cited.

A second cause of depressed mitogen reactivity in lung cancer may be intrinsic defects of the lymphocytes. Smetana, Vlastiborova, Matejkova et al. (1976) have found increased numbers of immature and stimulated lymphocytes in peripheral blood from lung cancer patients. It may be that these cannot respond normally to mitogens. Even where the polyclonal mitogen reactivity is found to be normal by conventional methods, lymphocyte function may be shown to be abnormal by newer techniques. Brzyski, Konchanin, Baustin and Ruckdescel (1979) divided the

lymphocyte proliferative response into nuclear-related events including ^{3}H-thymidine uptake and membrane-related events which consisted of transformation and blastogenesis. Using flow-cytometry, they found that, in patients with normal ^{3}H thymidine uptake on stimulation by PHA., lymphocyte transformation and blastogenesis were significantly reduced. Colchicine and cytochalasin C could mimic the effect of lung cancer when added to lymphocytes from normal donors. These drugs impaired the microtubule-microfilament related surface modulating assembly of the lymphocyte. This may be one of the mechanisms whereby lymphocyte function is impaired in lung cancer.

In a similar vein, Whitcomb and Parker (1977) found that, although PHA. reactivity in newly diagnosed lung cancer patients was similar to that of controls, PHA-stimulated protein synthesis by the lymphocytes was significantly reduced. In vitro lymphocyte DNA synthesis was normal and the discrepancy was not explained by reduction in T cell numbers.

The finding of depressed lymphocyte transformation by PPD. with normal transformation by PHA. and PWM. has not been reported by other workers. As cutaneous tuberculin reactivity was normal in these patients (see later) it suggests that, whereas the sensitised lymphocytes recognised the antigen normally and were able to initiate the appropriate inflammatory response, their transformation was inhibited by some other factor. Again suppressor cells may be the culprits. BCG. in vivo increased

suppressor cell activity (Jerrells *et al.*, 1979) so it is possible that tuberculoprotein (PPD. of tuberculin) enhances suppressor cell activity *in vitro*.

Rees, Rossio, Wilson *et al.* (1975) also found a discrepancy between PHA. and PPD. reactivity but in the opposite direction. In their study there was some correlation between the tuberculin test and lymphocyte stimulation by PPD.. Moreover PHA. and PPD. reactivity were found to correlate well in a study of normal subjects receiving BCG. vaccination reported by Thomas, Coy, Lewis and Yuen (1971).

Delayed hypersensitivity skin tests

In vivo testing of cell-mediated immunity in humans with lung cancer has taken the form of testing skin reactivity to recall or new antigens. Recall antigens are those to which the subject is likely to have been exposed previously; new antigens are those which have not been encountered before. These can be classified as follows:

Recall antigens	
Bacterial	Tuberculin (PPD., Old Tuberculin) Streptokinase/Streptodornase (Varidase)
Viral	Mumps
Fungal	*Trichophyton* *Candida albicans*
New antigens	
	DNCB.
	PHA.
	Keyhole limpet haemocyanin (KLH)

In our study tuberculin was chosen because it was readily available and because the author had experience of its use in other studies. DNCB. had already been used in one of the hospitals where these investigations were taking place.

From Table 16, it can be seen that 2 of 3 studies in the literature found no depression of tuberculin reactivity in patients with localised lung cancer and this was in agreement with the findings in this investigation both for mean tuberculin reaction (Table 8) and for the incidence of positive tuberculin tests (Figure 21). The exception was the report of Israel et al. (1973) in which the incidence of positive tuberculin tests in controls was taken from a population survey. They used 3 units of Pasteur PPD. which produced > 5 mm induration at 72 hours in 80% of adult males in an urban population. No mention of age-matching was made and it seems doubtful whether such a high incidence of reactivity would be found in controls who were matched for age and smoking habits. In contrast we used 10 units of PPD. (Weybridge) which produced a reaction of more than 10 mm at 48 hours in 68% of controls matched for age, sex and smoking habit who were also taken from a population survey in an urban area (Hawthorne, personal communication).

Holmes and Golub (1976) found that virtually every case with resectable lung cancer became sensitised after one exposure to DNCB.. They did not use controls but, of the 3 groups of workers who did, only one confirmed our finding of depressed prevalence of DNCB. reactivity

Authors		Controls	Recall antigens	DNCB.
Localised	Jansen et al. 1979 [a]	Normal and COAD matched for age, sex and smoking	=	↓
	Israel et al. 1973	Adult male urban population	↓	-
	De Meester et al. 1979	Benign pulmonary disease patients	=	=
	Wanebo et al. 1976	Benign pulmonary disease patients	-	=
Unselected	Krant et al. 1968	Age matched stroke patients	↓	↓
	Inoue et al. 1978	Benign pulmonary disease patients over 40	-	↓
	Szczepaniec and Pieton, 1979	Healthy subjects and non-malignant pulmonary disease patients	↓	-

Table 16. Delayed hypersensitivity skin tests in lung cancer patients with localised disease and in unselected series of patients.

= same level of reactivity
↓ depressed reactivity
- not done

in localised lung cancer (Jansen et al. 1979). This discrepancy cannot be explained by the use of matched controls as in general these tend to have a higher smoking incidence and to be older than unselected healthy controls drawn from hospital and laboratory staff, blood donors and volunteers.

Wanebo et al. (1976) claimed decreased incidence of DNCB. sensitisation in all stages of lung cancer. However, although 20 out of 20 normal subjects became sensitised to this antigen, as many as 29 out of 35 lung cancer patients between stages 0 and 2 were also sensitised. Failure of 6/35 to become sensitised can hardly be considered to represent overall depression of sensitivity to this antigen.

In vivo DNCB. reactivity in lung cancer correlated well with in vitro lymphocyte transformation in mixed lymphocyte culture (Holmes and Golub, 1976). Both tests measure the ability to recognise foreign material (hapten and allogeneic lymphocytes respectively) and the ability to respond to these. In patients already possessing lymphocytes sensitised to tuberculoprotein, the tuberculin test measures only the ability to respond to antigen. Decreased DNCB. reactivity coupled with normal tuberculin reactivity is therefore taken to indicate impairment of the afferent limb of the immunological response (Holmes and Golub, 1976) (Figure 22).

Generalised depression of immunological tests has been found in more advanced lung cancer (Table 16). In

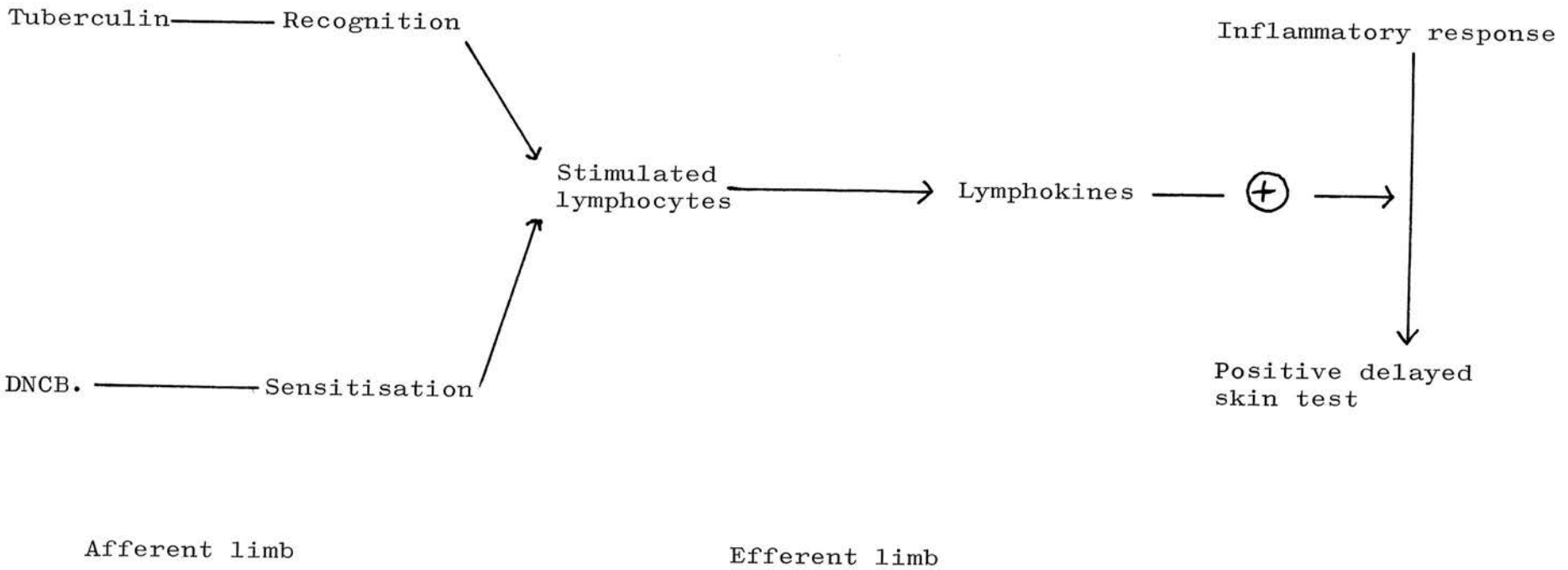


Figure 22. Delayed hypersensitivity skin reaction to tuberculin and DNCB.

some earlier studies, no correlation was found between DHS. tests and lymphocyte transformation tests (Golub, O'Connell and Morton, 1974; Saumon, Dermenghem, Saint-Paul et al., 1968). However in later studies, DHS tests have correlated with laboratory tests of immunological function (lymphocyte migration inhibition by allogeneic C14 cell line antigen, Cerni and Mickshe, 1976; leukocyte migration index, Pouillart et al., 1976). In our own study the striking relationship was between DNCB. reactivity and lymphocyte transformation by PPD.. As the former tests the afferent and effector limbs of the immunological response and the latter, only the effector limb, this finding argues in favour of a more generalised depression of immunological function.

DHS. tests using tumour extracts were first employed by Stewart (1969). Positive reactions were found in 4 out of 9 lung cancer patients.

Two mechanisms of depression of DHS. reactivity in lung cancer have been postulated. The first is inhibition by factors present in the serum (Golub et al., 1974). This was suggested by the close correlation of DNCB. reactivity with the mixed lymphocyte culture transformation test which was markedly inhibited by sera from lung cancer patients. However if this were the only mechanism, DNCB. and KLH. reactivity would be expected to disappear at the same time as inhibitory migratory activity of serum rather than before as found by Pouillart et al. (1976).

A second mechanism is depression of cell-mediated immunity by endogenous cortisol. De Meester, Golomb, Dudek et al. (1979) found an inverse relationship between the degree of DHS. reactivity and the midnight serum cortisol as the disease progressed. The hypothesis was tentatively formed that elevated serum cortisol was responsible for decreased skin and lymphocyte reactivity. However in the discussion following this paper it was pointed out that even the peak concentrations of cortisol encountered in these patients had no effect on mitogen-induced lymphocyte transformation in vitro.

Immunological tests before operation and the stage of the tumour

In general, cell-mediated immunity becomes depressed with progression of lung cancer. This study has shown that total lymphocytes, B cell count and DNCB. reactivity were reduced in stage 3 cases compared to stage 1 cases. Serial measurements of total lymphocytes in lung cancer have shown a fall with progression (Anthony et al., 1975) and Wanebo et al. (1976) showed that significantly more patients with stage 3 disease had total and T lymphocyte counts below the 10th percentile of control patients. B cell depletion in advanced disease has not been reported. However it is now recognised that the method used in our study may have counted a substantial number of monocytes and T cells.

DHS. reactivity to recall and new antigens has also been depressed in stage 3 squamous cell carcinoma (Jansen

et al., 1979a). Ruszel et al. (1978) found positive reactions to DNCB. in 51% of all lung cancer patients but in only 33% of patients with advanced disease. Similarly depressed lymphocyte transformation ratios have been found in some series (e.g. Giuliano et al., 1979) though not in our own cases. It is of interest that, during the evolution of lung cancer, DHS reactivity to DNCB. or KLH. disappeared before reactivity to recall antigens (Pouillart et al., 1976).

Immunological tests before operation and histology

In this study tuberculin reactivity was significantly greater in patients with large cell anaplastic carcinoma and adenocarcinoma than in those with squamous cell carcinoma (Table 11). Jansen et al. (1979a) found greater depression of DHS. and laboratory tests of cell-mediated immunity in squamous cell carcinoma. In particular, there was a greater incidence of negative tuberculin tests in patients with squamous cell carcinoma stage 3 than in patients with adenocarcinoma and small cell carcinoma. Further indirect evidence of more pronounced suppression by squamous cell carcinoma comes from Dellon et al. (1979) who found that depression of total and T lymphocytes in this tumour but not in adenocarcinoma indicated a bad prognosis.

Most other comments on the immunological activity of different histological types group adenocarcinoma and squamous cell carcinoma together and contrast them with anaplastic tumours including small cell carcinoma. For

example Weese, Oldham, Herberman et al. (1976) found that depression of T cell counts and lymphocyte transformation tests was greater in patients with anaplastic tumours, a finding confirmed for T cells by Huang, Yang and Rafla (1978). Similarly Ioachim, Dorsett and Paluch (1976) found increased plasma cells and lymphocytes in squamous cell carcinoma compared with undifferentiated and small cell carcinoma tissue. As small cell carcinoma patients were deemed unsuitable for surgery and therefore excluded from our series, I cannot comment on the relative immunological behaviour of these from our own results. However it seems that immunological depression may be even more pronounced with these tumours than with squamous cell carcinoma.

Immunological tests before operation and clinical progress after operation

This investigation has shown that total lymphocyte counts and DNCB. reactivity were the only tests which correlated significantly with prognosis. The prognostic value of total and T lymphocytes was described by Dellon et al. (1979). They found that all patients with tumours other than adenocarcinoma who had total lymphocytes less than 1000/cu.mm. and T cells less than 750/cu.mm. died within 3 months whereas 55% of those with higher levels were surviving and free from clinical evidence of tumour recurrence over nine months later. The prognostic significance of total lymphocyte count was confirmed for stage 3 tumours by Wanebo et al.,(1976)and of T cell counts by Anthony et al. (1975).

The prognostic value of the tuberculin test in operable lung cancer was reported by Israel et al. (1973). Since then there have been numerous reports of the relationship between DNCB. reactivity and prognosis. Concannon, Dalbow, Davis et al. (1978a) built up a profile of immunological tests in patients referred for radiotherapy and found that the DNCB. test was the best predictor of survival. De Meester et al. (1979) found a parallel fall in the percentage of patients with a positive reaction and the current median survival. Liebler et al. (1977) found that depression of DNCB. reactivity was associated with decreased survival at all stages of the disease. The only contrary report was that of Wanebo et al. (1976) who found no significant difference in survival between DNCB. positive and DNCB. negative patients in each stage of the disease.

In our investigation, lymphocyte transformation studies did not correlate with clinical results during 2 years after operation. This confirms the findings of Braeman and Deeley (1973) for PHA. and PPD. In contrast, Wanebo et al. (1976) found PHA-induced lymphocyte transformation a useful prognostic test in stage 3 carcinoma. An extension of this observation was the discovery that patients whose serum had a strong inhibitory action on PHA. and Con A transformation of normal donor lymphocytes had a poorer prognosis than patients whose serum had little inhibitory effect (Giuliano et al., 1979).

In general it can be said that, in an individual patient, generalised depression of a battery of immunological tests is likely to indicate a poor prognosis but that the variability of results is such that any single result taken alone is not of great prognostic value.

CHAPTER 7

IMMUNOLOGICAL EFFECTS OF IMMUNOTHERAPY

Results

In Table 17, it can be seen that there was no substantial difference in mean readings of any of the measurements made before operation between the autograft and non-autograft groups. None of the differences recorded reached significant levels and the overall base line immunological function of both groups was thus similar.

In Figures 23 to 27, changes in the various measurements from the pre-operative measurement were recorded during the post-operative period. In both groups there was a significant rise in total w.b.c. during the 3 weeks after operation, and a rebound fall which reached significant levels ($p < 0.05$) only in the autograft group at 8, 14 and 17 months (Figure 23). Total lymphocytes had fallen significantly in both groups at 1 week after operation and there were isolated falls at 8 months (non-autograft) and 17 months (autograft) (Figure 24). T cells followed a similar pattern during the immediate post-operative period although the mean fall in the control group was not significant ($p > 0.05$) (Figure 25).

As mentioned in the previous section, there was extreme variation in the lymphocyte transformation readings among and between individual patients. Thus although a definite pattern of post-operative depression followed by recovery of PHA-induced lymphocyte transformation was seen in both groups (Figure 26) the change from the base line median reading was not found to be significant by

	Autograft group	Non-autograft group	
Total w.b.c.	8823	8600	per cu.mm.
Total lymphocytes	2397	2346	per cu.mm.
Total T cells	999	830	per cu.mm.
Percentage T cells	36	36	%
Total B cells	736	568	per cu.mm.
Percentage B cells	25	25	%
Lymphocyte transformation ratios			
PHA.	151	116	
PWM.	38	27	
PPD.	1.6	2.5	
Delayed hypersensitivity skin tests			
Tuberculin test	14	18	mm.
Percentage positive DNCB. test	37	23	%

Table 17. Mean pre-operation values for immunological tests and percentage positive DNCB. reactors in the main trial autograft and non-autograft patients. There are no significant differences between the two groups.

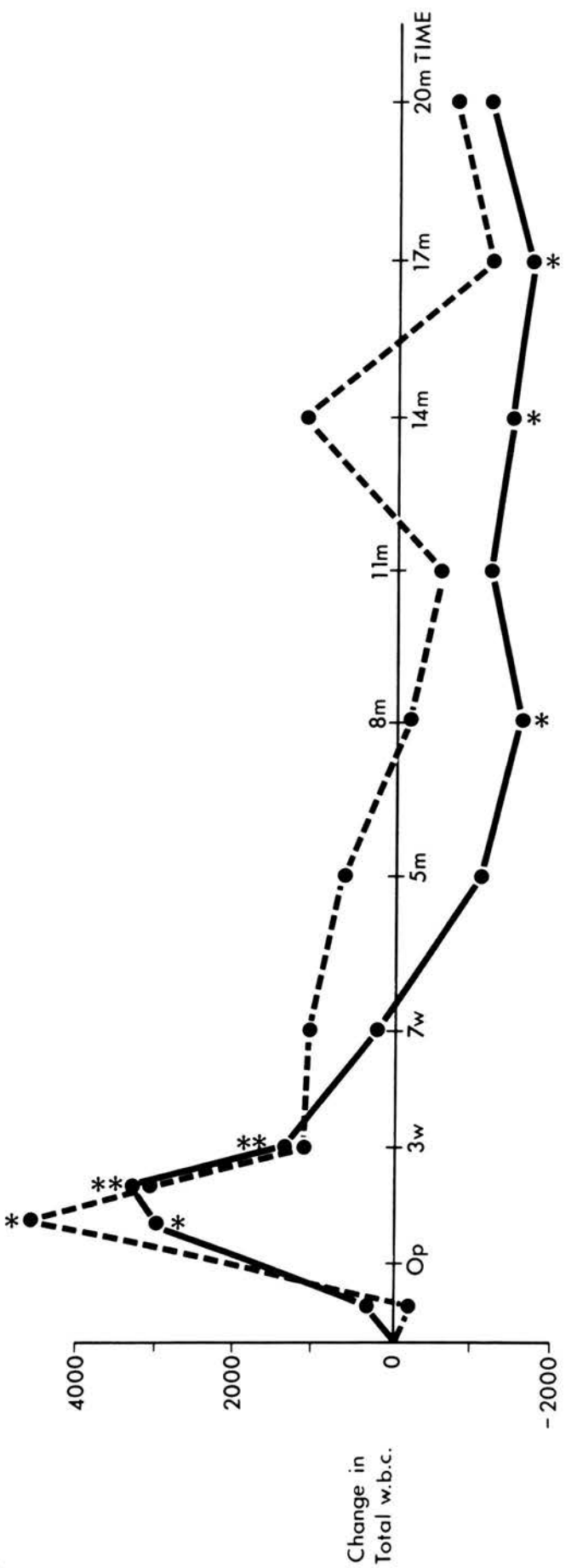


Fig. 23 Mean change in total w.b.c. per cu.mm. after operation in main trial autograft (solid line) and non-autograft (interrupted line) groups. Significant change ($p<0.05$) indicated by asterisk.

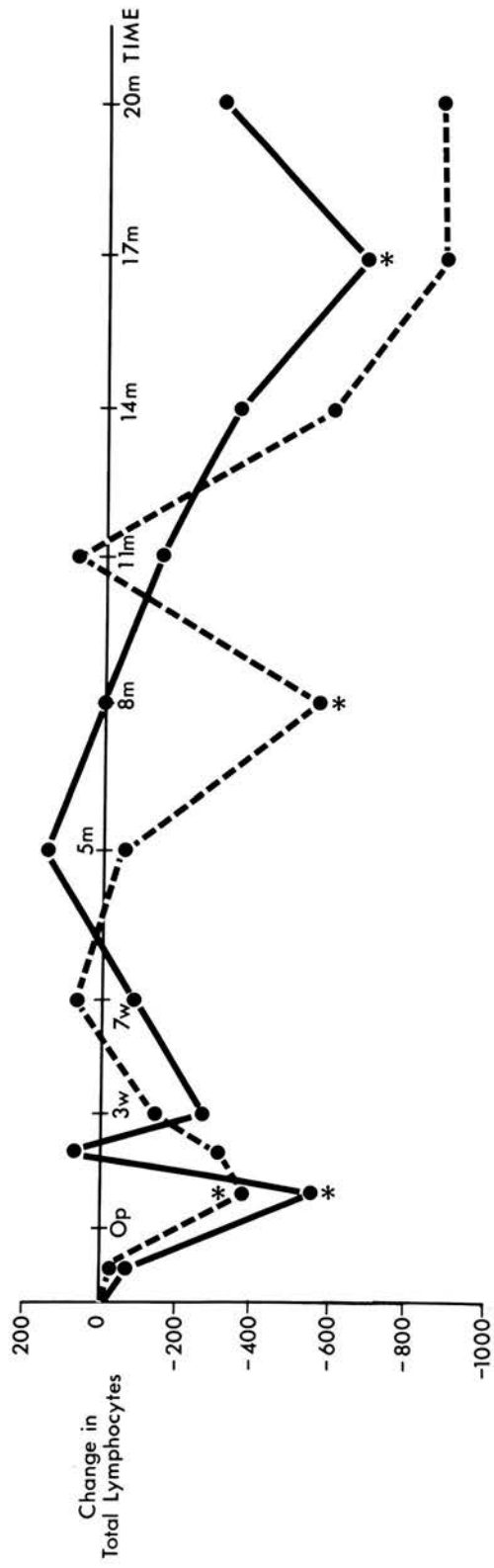


Fig. 24 Mean change in total lymphocytes per cu.mm. after operation in main trial autograft (solid line) and non-autograft (interrupted line) groups. Significant change ($p < 0.05$) indicated by asterisk.

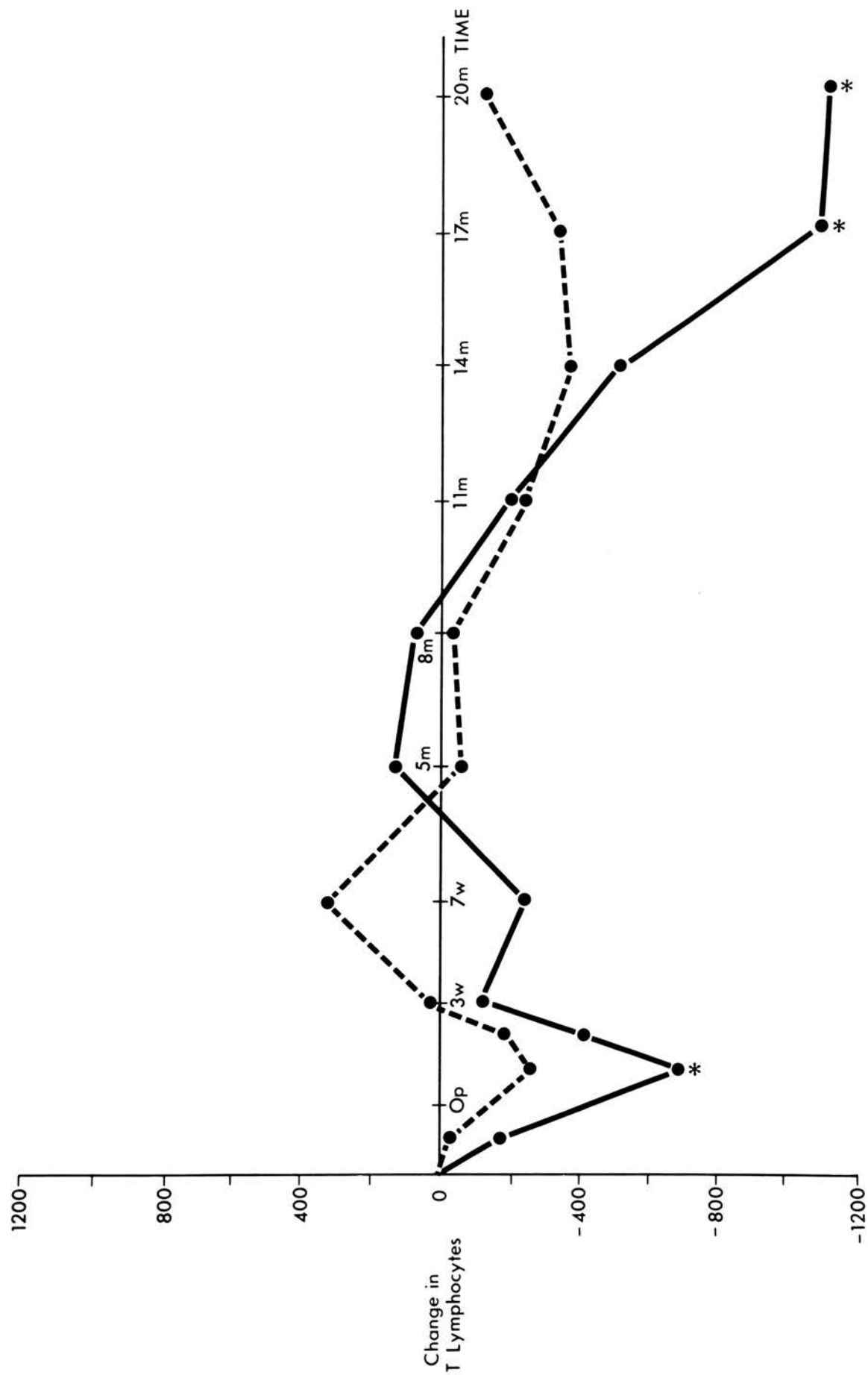


Fig. 25 Mean change in T lymphocytes (per cu.mm.) after operation in main trial autograft (solid line) and non-autograft (interrupted line) groups. Significant change ($p < 0.05$) indicated by asterisk.

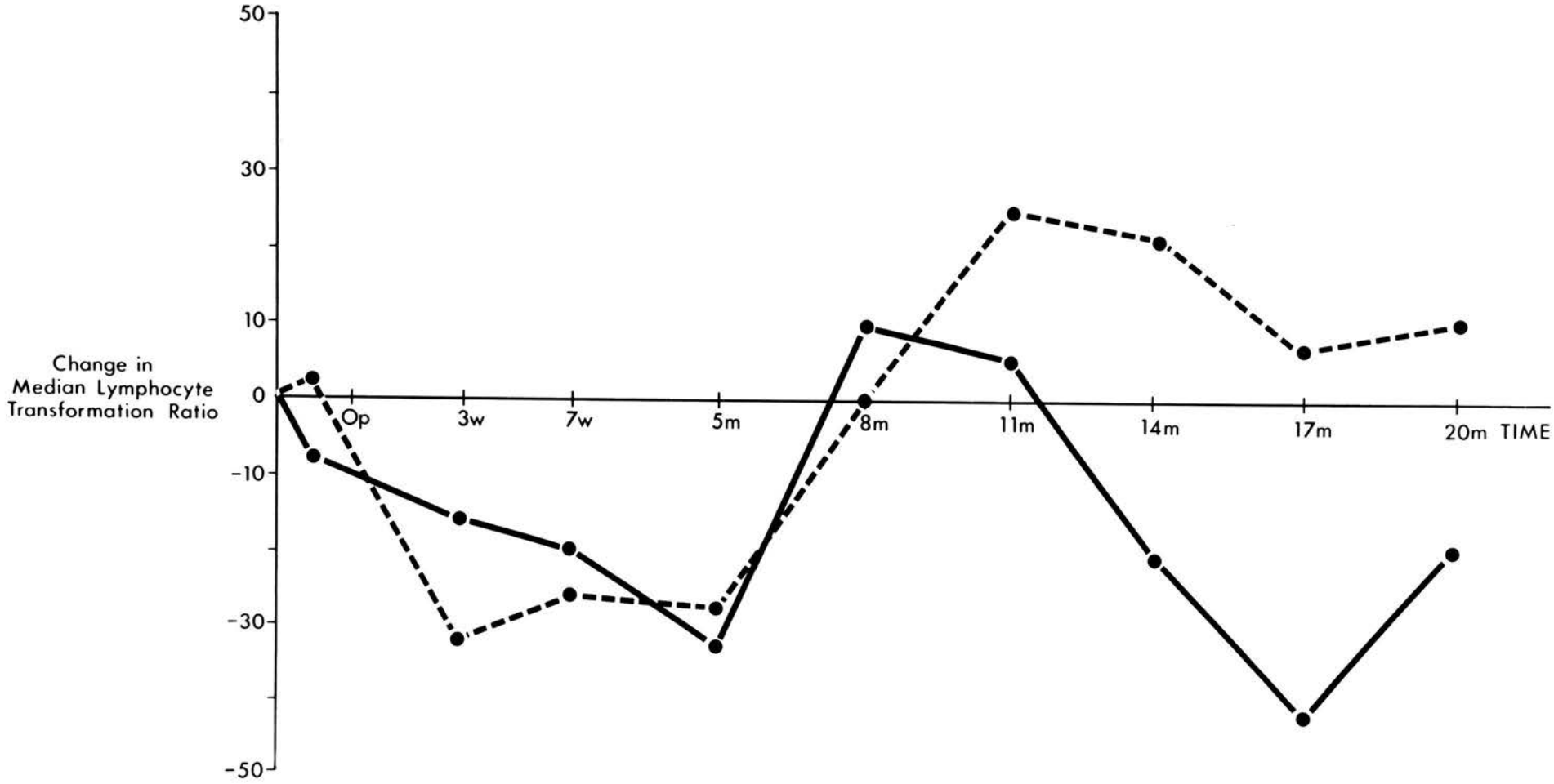


Fig. 26 Mean change in median lymphocyte transformation ratio with P.H.A. after operation in main trial autograft (solid line) and non-autograft (interrupted line) groups.

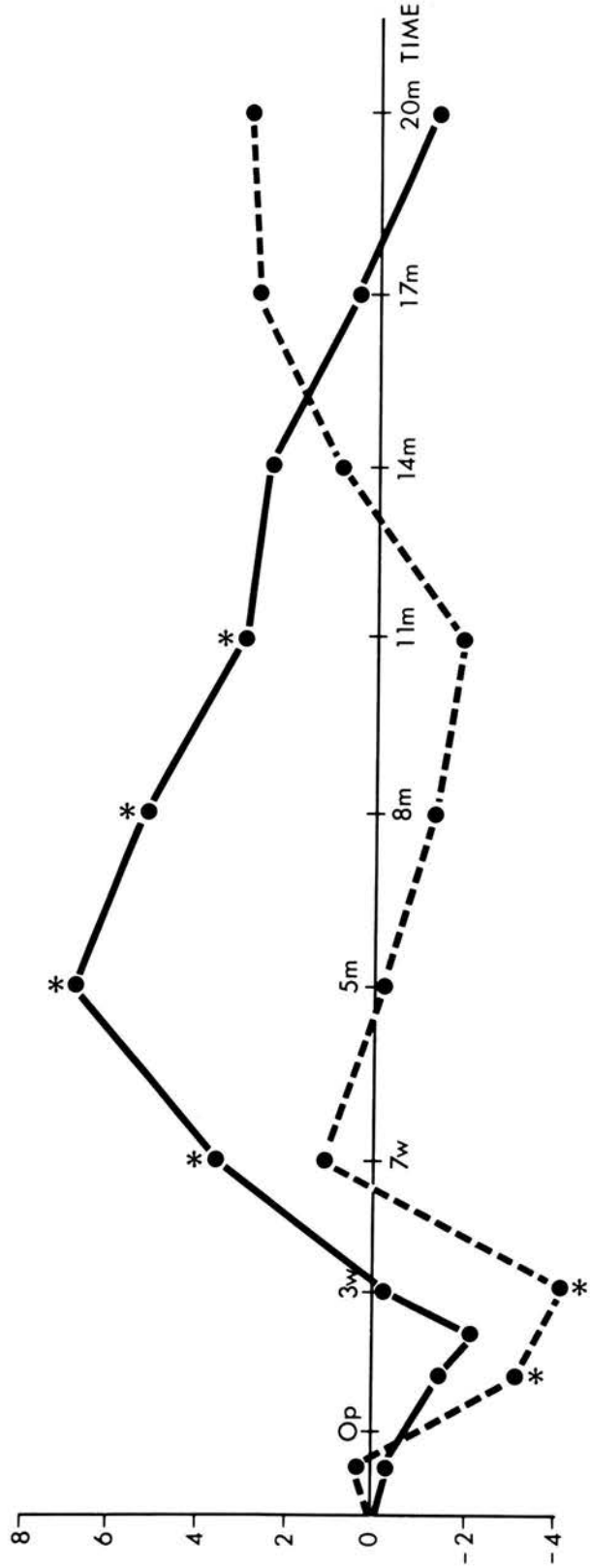


Fig. 27 Mean change in maximum diameter of tuberculin reaction at 48 hrs. in main trial autograft (solid line) and non-autograft (interrupted line) patients after operation. Significant change ($p < 0.05$) indicated by asterisk.

the statistical methods used. Similarly there were no significant changes in lymphocyte transformation ratios for PWM. and PPD.

As might be expected, much the most meaningful results were obtained in the change from base line measurements of the tuberculin test (Figure 27). The mean values fell during the immediate post-operative period though only the fall in the non-autograft group was significant. A subsequent rise in the autograft group was seen at 7 weeks and at 5, 8, 11 and 14 months. There was no significant rise in tuberculin reactivity in the control group. The mean DNCB. reactivity (Table 18) increased in both groups at 3 and 7 weeks after operation.

Discussion

(a) Immediate post-operative results

One of the problems in assessing the value of immunotherapy in lung cancer from published reports is that workers have frequently claimed clinical success for their treatment without producing any evidence of an immunological effect (Djurovic and Decroix, 1978; Miyazawa, Suemasu, Ogata et al., 1979; Hadziev, Kavaklieva-Dimitrova, Mandulova et al., 1980). One reason for the omission of these important data is an intention to publish the immunological results elsewhere (e.g. Edwards and Whitwell, 1974). Another is the misguided belief that, as the immunological results do not appear to correlate with other findings, they are better omitted altogether. A third reason is that the shortage of time available at

Patients	Postoperative increased DNCB. reaction	
	3 weeks	7 weeks
Autograft	0.5	1.0
Non-autograft	0.5	1.7

Table 18. Skin reactivity to DNCB. at 3 and 7 weeks after operation.

scientific meetings means that clinical results are included in preference to immunological findings in the papers given and in their abstracts.

Another drawback in this field is the absence of more specific tests. We know that killing of tumour cells by macrophages and lymphocytes is an important part of the host defence against tumours and that immunological stimuli act on these immunologically competent cells. Yet specific tests of macrophage activity and tumour cell killing are time consuming and difficult to perform. In our own study macrophage migration inhibition tests had to be abandoned because of lack of laboratory time available. This is a field where further progress is needed. Some attempts have already been made including the demonstration of increased lymphocyte cytotoxicity against tumour cells in patients treated with BCG. - CWS., referred to by Yasumoto, Manabe, Yanagawa et al. (1979). A more recent report of a modified skin window technique to measure non-specific macrophage chemotaxis also holds promise (Israel, Samak, Bogucki and Samak, 1981).

In this investigation of immunotherapy before and after surgery, it is right to bear in mind that the major immunological event in the immediate peri-operative period is thoracotomy and resection of the tumour under general anaesthesia rather than the immunotherapy that was given. Immunological effects of surgery have been under investigation for some years. Wingard, Lang and Humphrey (1967)

found a fall in total w.b.c. and lymphocytes in rats receiving intraperitoneal sheep red blood cells who were given halothane. Lundy, Lovett, Hamilton and Conran (1978) reported that in mice with methyl cholanthrene-induced fibrosarcoma surgery and anaesthesia but not anaesthesia alone impaired cell-mediated cytotoxicity and increased pulmonary metastases. The effect of surgery, radiotherapy and nutrition on immunological function has more recently been reviewed by Ota, Copeland, Corriere and Dudrick (1979).

In our study a rise in total w.b.c. was seen in both groups during the first 3 weeks after operation. Cullen and van Belle (1975) showed that this increase was proportionate to the degree of trauma involved but did not vary with different anaesthetic agents employed. Catecholamine release was thought to be a major factor. In their study, total lymphocytes increased slightly after operation. However Slade, Simmons, Yunis and Greenberg (1975) showed that in normal volunteer kidney donors, total, T and B lymphocytes fell during operation. Return to normal values occurred between 2 and 6 days after operation.

Several authors have found depressed lymphocyte transformation by mitogens after surgery. This occurred within a few hours of operation (Park, Brody, Wallace and Blakemore, 1971) and returned to normal within a week. There was slight variation in the extent and pattern of response to different mitogens (Berenbaum, Fluck and Hurst, 1973) but the prolonged fall in both groups seen in our series was not described (Figure 26).

Bancewicz, Gray and Lindop (1973) reported that all 9 of their tuberculin positive patients became tuberculin negative after surgery and general anaesthesia and remained so for 9-12 days. Endogenous cortisol release has been considered a possible factor in the depression of lymphocyte reactivity to mitogens (Cullen and Van Belle, 1975). However Berenbaum et al. (1973) showed that incubation of peripheral blood leucocytes with cortisol over 16 hours did not reduce their response to PHA.. They also found that reactivity to PHA. of lymphocytes from post-operative patients was depressed even when their plasma cortisols had been normal. In our control group, this depression of tuberculin reactivity was clearly seen whereas the percutaneous BCG. given at the time of operation and twice thereafter seemed to protect the autograft group from this effect.

(b) Long term immunological results

The post-operative depression of lymphocyte transformation by PHA. and its subsequent recovery is illustrated in Figure 26. Some reports of lymphocyte transformation by mitogens in lung cancer patients treated with immunotherapy are summarised in Table 19. It can be seen that the results are rather variable. In the only other study where specific immunotherapy was employed (Oldham, Weese, Herberman et al., 1976) there was no change in reactivity to any of 3 mitogens.

The discrepancy in lymphocyte transformation results between different workers can partly be attributed to the

Authors	Patients	Systemic Immunotherapy	Mitogen	Effect on Reactivity
Yasumoto et al., 1979	Stage 1,2 and 3 patients, some after surgical resection	BCG. - CWS.	PHA.	Increased
Robinson et al., 1977	Post-surgical, radiotherapy or chemotherapy, majority with locally advanced disease and/or metastases	MER. of BCG.	PPD. PHA.	Increased No significant change
Jansen et al., 1978	Locally advanced squamous cell cancer treated by resection	BCG.	PPD.) Con A) PHA.	Increased at 1 year No change
Gross and Eddie-Quartey, 1976	Following surgery and/or radio-therapy	BCG.	PPD. PHA.	Increased Increased in tumour free
Kerman and Stefani, 1978	Inoperable locally advanced treated with radiotherapy	BCG.	PHA.	Increased
Oldham et al., 1976	All stages, treat-ed with surgery, radiotherapy or chemotherapy	BCG. or BCG. + allogeneic cells	PHA.) Con A) PWM.)	No change

Table 19. Lymphocyte mitogen-induced transformation tests after immunotherapy; summary of some published reports.

variable nature of the patients studied. Hence PHA reactivity increased in lymphocytes from patients with little or no tumour burden (Yasumoto et al., 1979; Gross and Eddie-Quartey, 1976) but not in some patients with locally advanced disease and/or metastases (Robinson, Bartal, Cohen et al., 1977). Contrary to our findings, lymphocyte transformation by PPD increased in the 3 groups tested who had received BCG.. Failure of this to occur in our study may have been due to the short period of time over which BCG. was given and to the fact that it was given so soon after operation when immunological function was depressed. Yet the expected increase in skin reactivity to tuberculin did ensue.

Some reports of the effect of non-specific immunotherapy on skin reactivity after resection of lung cancer are summarised in Table 20. Levamisole is an immunorestorant. As tuberculin reactivity is not generally depressed in operable lung cancer, it is not surprising that this drug did not increase reactivity to tuberculin. However in all series where BCG. was given, there was increased mean tuberculin reactivity, conversion of negative to positive reactors or increased incidence of positive reactors during the post-operative period. These measurements were made within 3 months of operation and an exact time pattern was not recorded. Our study showed that immunotherapy incorporating percutaneous BCG. did produce increased skin reactivity between 7 weeks and 11 months after operation. Thus the immunological benefit began to wane at a time when the

Authors	Patients	Immunotherapy	Effect on skin reactivity
Jansen et al., 1978	Resected squamous cell carcinoma, stage 2	Percutaneous BCG.	PPD. ↑ DNCB. ↑
McKneally et al., 1976a	Resected lung cancer, different stages and histology	Intrapleural BCG.	PPD. ↑
Amery, 1980	Resected lung cancer, different stages and histology	Levamisole	PPD. = DNCB. =
Wright et al., 1980	Resected non-small cell lung cancer	Intrapleural BCG.	PPD. ↑

Table 20. Some published reports of the effect of immunotherapy on skin reactivity to PPD. and DNCB..

advantage in freedom from tumour recurrence became less clear cut.

Relatively few of the reports reviewed involved specific immunotherapy. However Oldham et al. (1976) reported an increase in reactivity to recall antigens in patients receiving immunotherapy some of whom were given allogeneic tumour cells and BCG.. Whether the addition of tumour cells or extract to the BCG. increases the tuberculin reactivity further is still unknown.

The finding of increased mean skin reactivity to DNCB. in both groups on testing 3 and 7 weeks after operation raises the question of whether repeated skin testing alone increases skin reactivity to recall or new antigens. Increased sensitivity to DNCB. has been reported on repeated testing of patients treated with Levamisole and control patients suffering from lung cancer of various stages by Holmes and Golub (1976). However Krant et al. (1968) found that 5/16 DNCB. positive patients became negative as the disease progressed to terminal stages.

Increased sensitivity to tuberculin on repeat testing has been reported by Richards, Nelson, Batt et al. (1979) in 6.6% of normal volunteers and by McKneally, Maver, Kausel and Alley (1976a) in 5/12 tuberculin negative control patients after successful resection of lung cancer. However repeated testing on up to 9 occasions did not produce an increase in the mean tuberculin reactivity of the non-autograft group in the present study. Moreover

the tuberculin reactivity of the autograft group waned gradually after 5 months following immunotherapy despite continued tuberculin testing. At least in these patients who had previously borne tumours, enhanced reactivity to tuberculin did not occur with repeated testing.

CHAPTER 8

CLINICAL RESULTS OF IMMUNOTHERAPY IN LUNG CANCER

Results

By dividing up the post-operative period into intervals of 3 months and recording the number of patients dying or developing tumour recurrence during each interval, it has been possible to construct actuarial life table curves for survival and time free of tumour recurrence in all patients and various subgroups (Figures 28 to 32). From these it has been possible to calculate the percentage of patients likely to survive or to be free of tumour recurrence at annual intervals during the first 3 years after operation and to derive the significance of differences between the curves (Tables 21 and 22).

Except for those with stages 2 and 3 tumours, the proportion of autograft group patients surviving and remaining free of tumour recurrence at any one time during the 3 years after operation was higher than that of the non-autograft group. However with these relatively small numbers of patients, the difference only approached acceptable levels of significance in stage I patients (Tables 21 and 22) and those who became sensitised to DNCB. before operation (Tables 23 and 24). In these groups only, injection of irradiated autologous tumour cells and BCG. may be effective.

From the graphs it is possible to detect a pattern in which the curves diverge at 6 months. In patients with stage I tumours, the difference becomes most marked at 18 months, remaining substantial up to 3 years. However when all patients are considered, the difference narrows

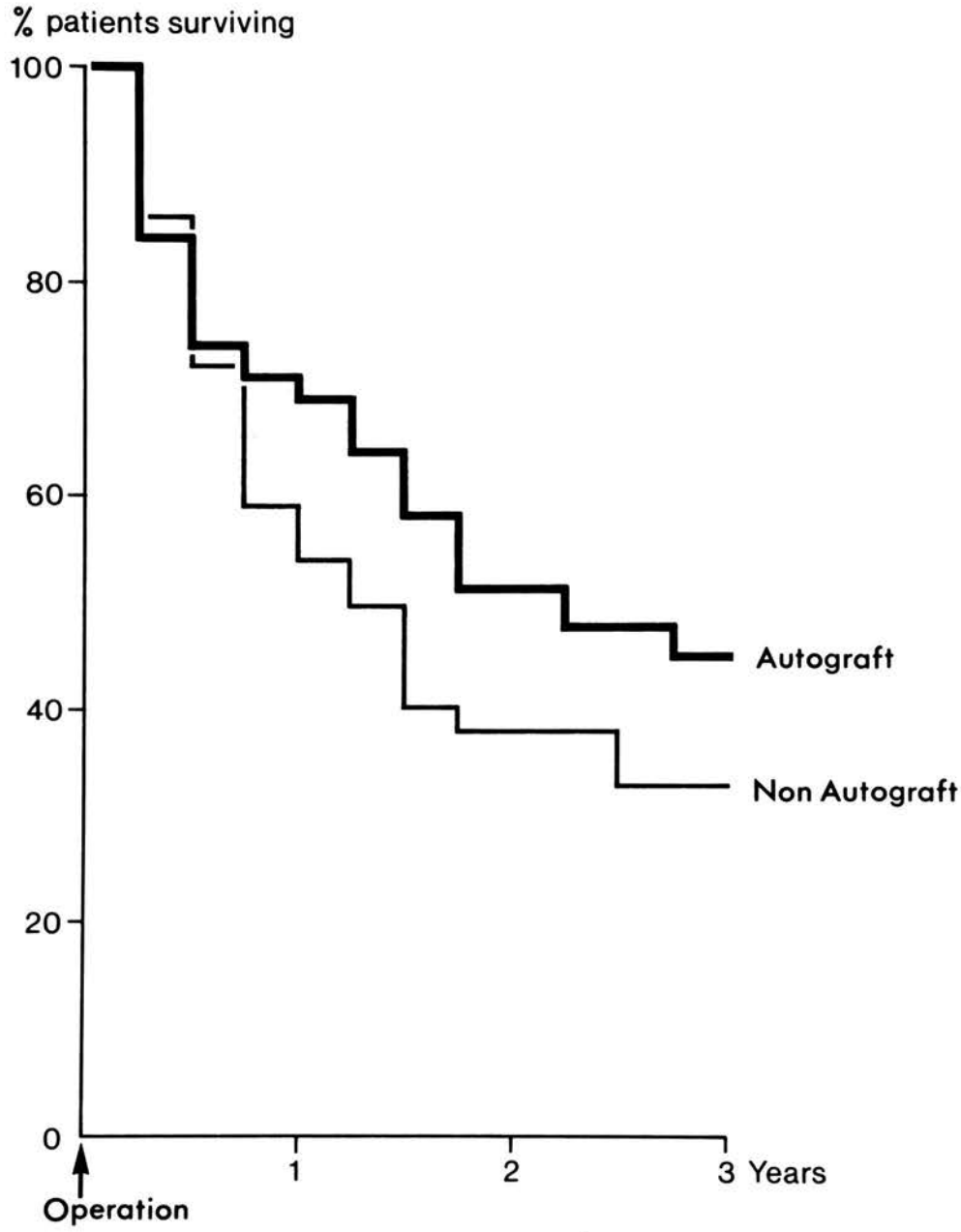


Fig. 28 Actuarially calculated survival graphs for all main trial patients according to treatment group. Difference not significant.

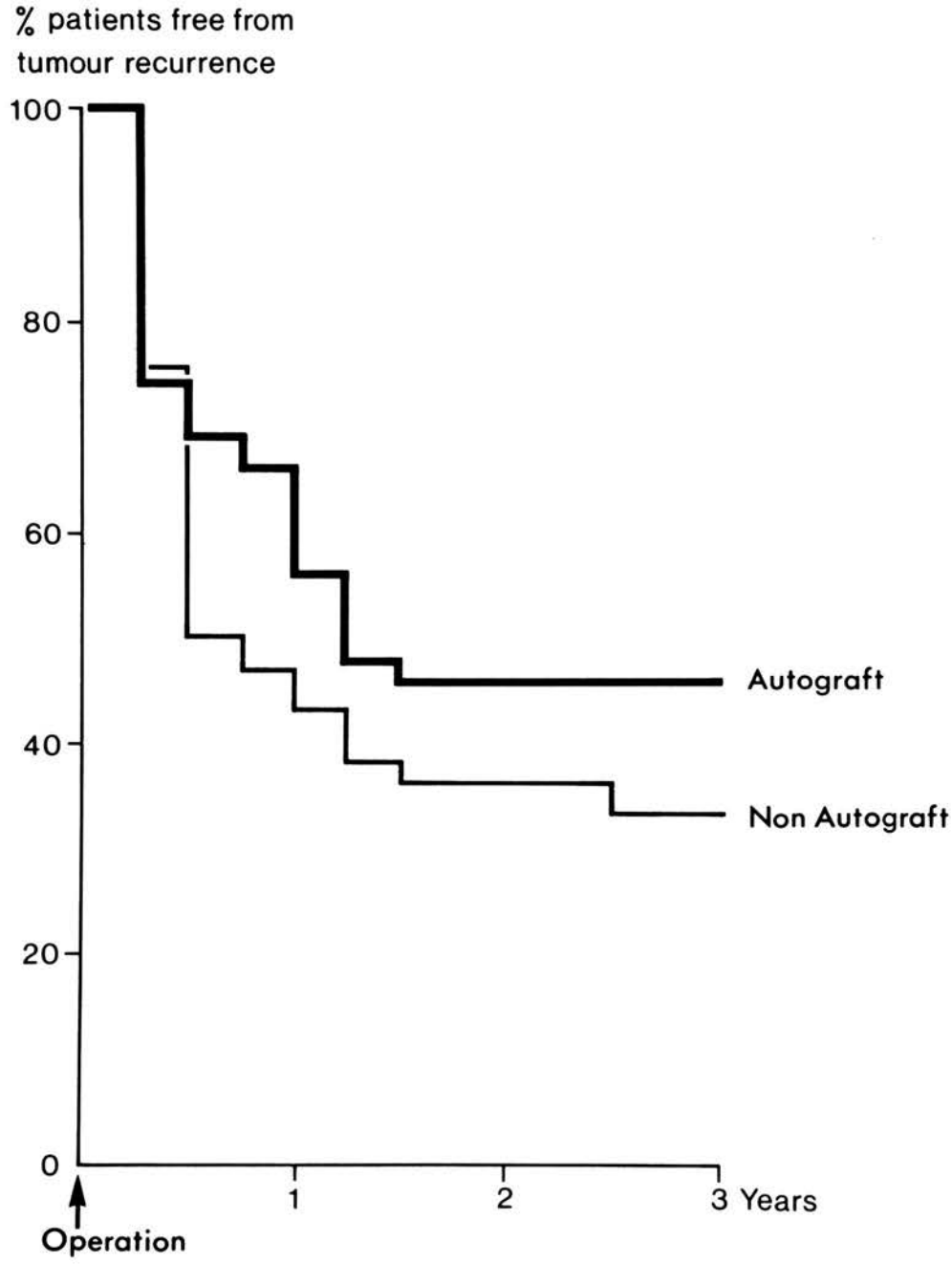


Fig. 29 Actuarially calculated graph for time of tumour recurrence of all main trial patients according to treatment group. Difference not significant.

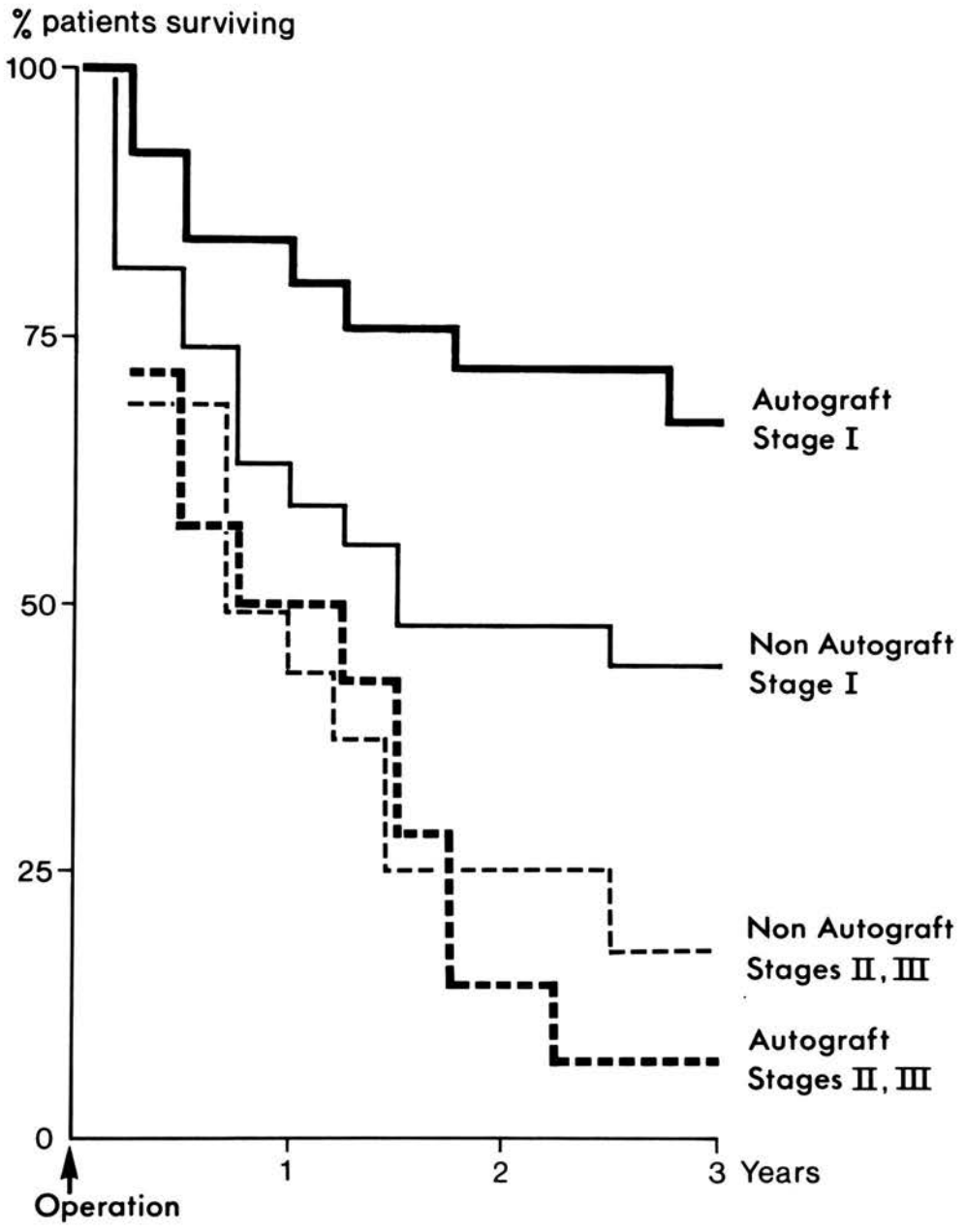


Fig. 30 Actuarially calculated survival graph of main trial patients according to treatment group and stage. For difference in stage I patients, $p = 0.09$.

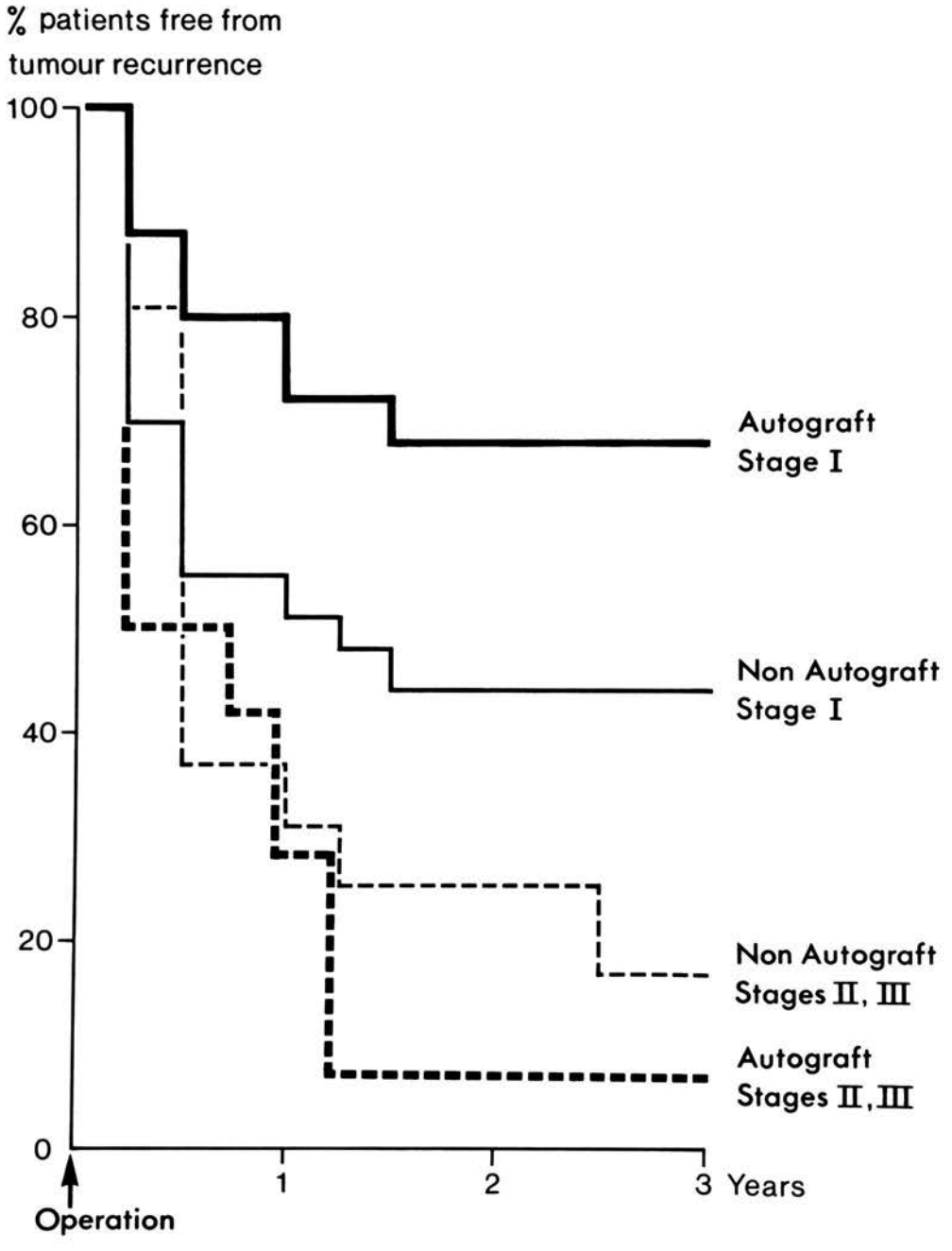


Fig. 31 Actuarially calculated graphs for time after operation in which main trial patients remained free from tumour recurrence, according to stage of tumour. For difference in stage I patients, $p = 0.09$.

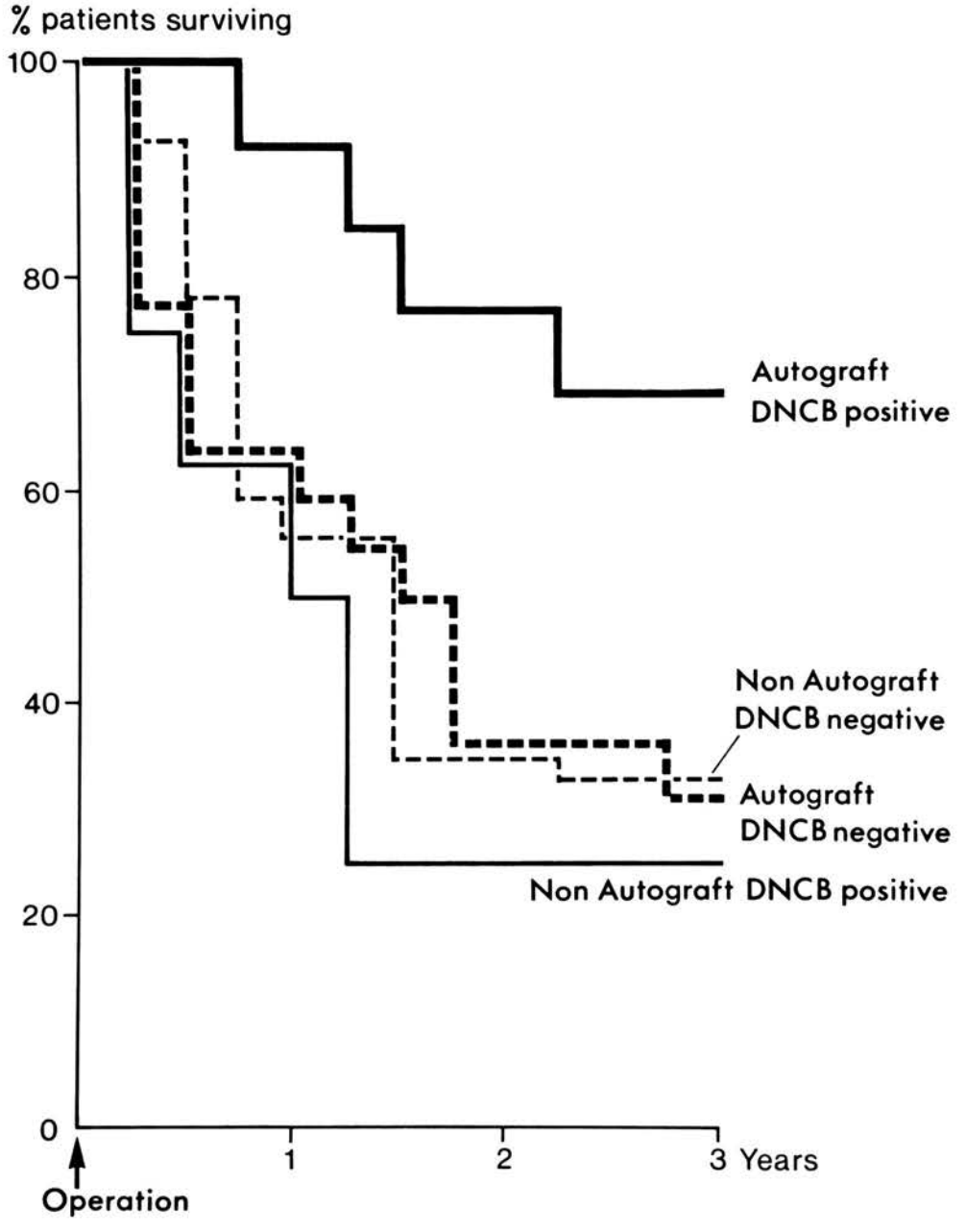


Fig. 32 Actuarially calculated survival graphs for main trial patients according to treatment group and DNCB reactivity before operation. Difference was significant, ($p = 0.02$) for DNCB positive cases.

		1 Year	2 Years	3 Years	Significance
All cases:	autograft	69	51	45	N.S.
	non-autograft	55	39	32	
Stage I:	autograft	76	72	67	p = 0.09
	non-autograft	55	48	44	
Stages II and III:	autograft	50	14	7	N.S.
	non-autograft	44	25	18	

Table 21. Percentages of patients likely to survive at 1, 2 and 3 years after operation derived from actuarially calculated curves.

	1 Year	2 Years	3 Years	Significance
All cases: autograft	56	46	46	N.S.
All cases: non-autograft	43	36	34	
Stage I: autograft	72	68	68	p = 0.09
Stage I: non-autograft	52	44	44	
Stages II and III: autograft	29	7	7	N.S.
Stages II and III: non-autograft	31	25	18	

Table 22. Percentages of patients likely to remain free of tumour recurrence at 1, 2 and 3 years after operation, derived from actuarially calculated curves.

	1 Year	2 Years	3 Years	Significance
DNCB. positive:				
autograft	92	77	69	p = 0.02
non-autograft	50	25	25	
DNCB. negative:				
autograft	59	36	32	N.S.
non-autograft	56	37	33	

Table 23. Percentages of patients likely to survive at 1, 2 and 3 years after operation according to pre-operation DNCB. reactivity.

	1 Year	2 Years	3 Years	Significance
DNCB. positive:				
autograft	77	69	69	p = 0.02
non-autograft	25	25	25	
DNCB. negative:				
autograft	45	32	32	N.S.
non-autograft	48	37	33	

Table 24. Percentages of patients likely to remain free of tumour recurrence at 1, 2 and 3 years after operation according to pre-operation DNCB. reactivity.

at 21 months, partly because non-autograft patients with stage 2 and 3 tumours did better than the autograft group after 21 months.

Median times for survival and freedom from tumour recurrence or progression have also been calculated for all patients in both groups, for different stages and histology (Tables 25 and 26). Here again the autograft group fared better in general but the difference was only significant at the $p = 0.07$ and $p = 0.08$ levels respectively in patients with stage I tumours.

As it is now more than 2 years since entry of the last patient into the trial, the number of patients actually alive and free from clinical and radiographic evidence of local or metastatic tumour 2 years after operation has been recorded (Table 27). Although the proportion was higher in the autograft group, especially in stage I patients, the differences were not significant.

The value of serial tests in detecting tumour recurrence

Because depression of DHS. tests and laboratory measurements of cell-mediated immunity is more marked in advanced lung cancer, it might be possible to detect tumour recurrence after operation by serial measurements. Indeed Shirakusa and colleagues (1978) showed that circulating T cell levels fell with recurrence of tumour. Figure 33 shows the individual measurements of lymphocyte transformation ratios for PHA. in patients at the time of tumour recurrence in relation to graphs of the mean change in ratios for the 2 groups. Nine out of 17 patients had

Patients	Autograft Group	Non-autograft group	Significanc
All	25	15	N.S.
Stage I	>36	17	p = 0.07
Stages II and III	9	9	N.S.
Squamous cell	>36	16	N.S.
Non-squamous cell	25	15	N.S.

Table 25. Median survival times (months) calculated for the first 36 months after operation.

Patients	Autograft Group	Non-autograft group	Significanc
All	14	6	N.S.
Stage I	>36	14	p = 0.08
Stages II and III	6	5	N.S.
Squamous cell	>36	10	N.S.
Non-squamous cell	13	5	N.S.

Table 26. Median time free from tumour recurrence (months) calculated for first 36 months after operation.

		No.	%
Autograft	All cases	18/40	45
	Stage I cases	17/24	71
Non-autograft	All cases	16/43	37
	Stage I cases	13/30	43

Table 27. Percentage of the patients in the autograft and non-autograft groups who were alive and free from clinical and radiographic evidence of tumour recurrence 2 years after operation. Differences not significant.

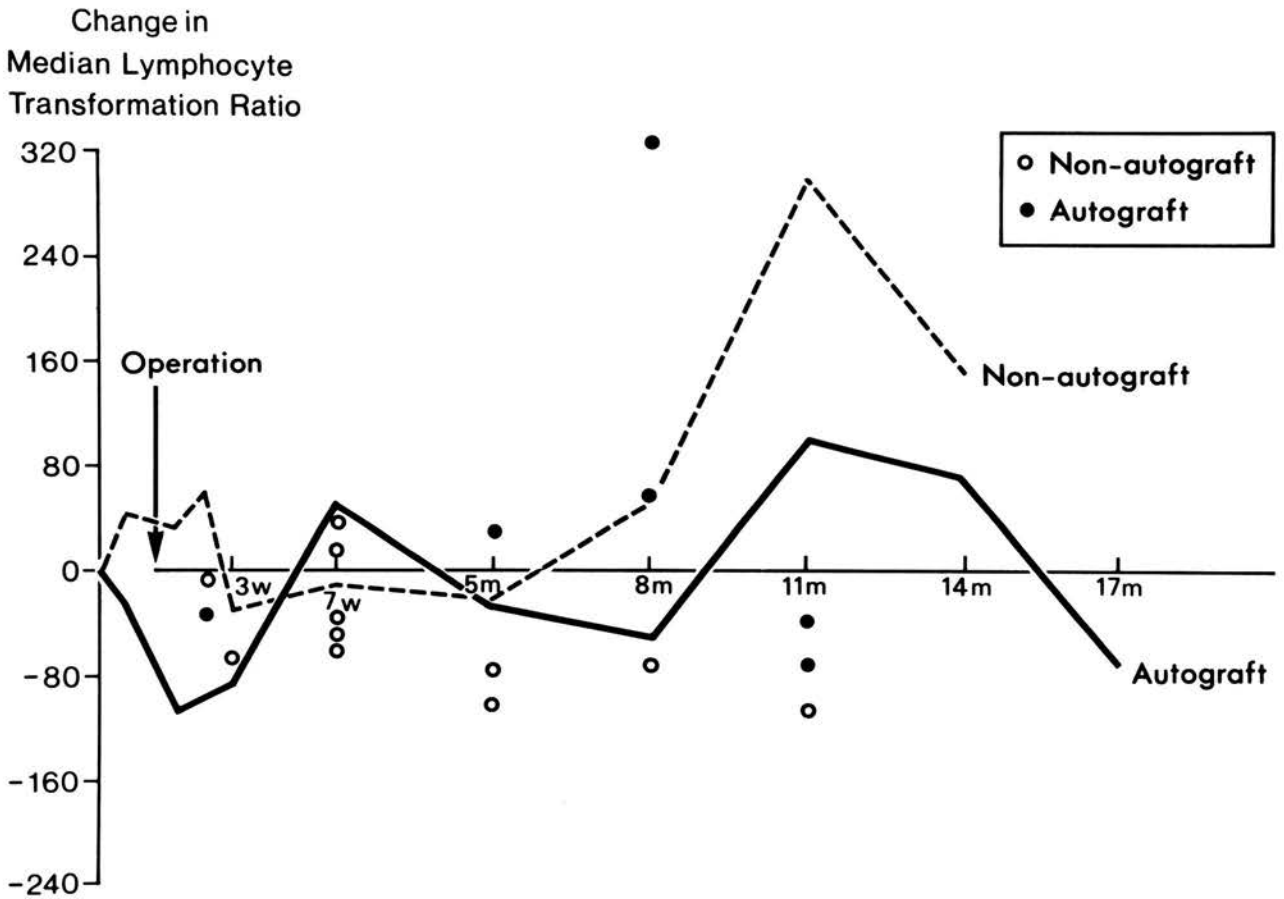


Fig. 33 Lymphocyte transformation ratio for PHA. of individual patients at the time of assessment prior to clinical detection of tumour recurrence in relation to the median lymphocyte transformation ratio for autograft (solid line) and non-autograft (interrupted line) groups.

values below the group mean at the time of recurrence and only one had a value above. Similar patterns were obtained for lymphocyte transformation ratios for PPD. and for total w.b.c., but not for circulating levels of total lymphocytes, T and B cells. Significant depression of one or more measurements of immunological function was common at the time of tumour recurrence but the pattern was too variable for serial measurements to be of clinical value.

Autopsies

Autopsies were performed in only 7 patients. This low autopsy rate was due to the wide catchment area served by the two participating hospitals. Thus many patients died at home or in their local hospitals. As the cause of death was usually seen to be recurrence of lung cancer, local physicians and surgeons did not press for autopsy examination.

Autopsy examination of 4 patients who received autologous irradiated tumour cells and BCG. did not reveal any evidence of local tumour growth at the site of injection. In none of these cases was there any evidence of granulomatous hepatitis, a recognised complication of BCG. therapy.

Discussion

In an earlier section (Chapter 3), I have described some of the research work that was published before this project started in 1975 and which influenced the design of the project. Since this investigation started, numerous other studies of immunotherapy in lung cancer have been undertaken. Progress in some of these has been reported

at regular intervals in published papers or abstracts of scientific meetings. Thus the volume of literature on the subject is now substantial. The methods of immunotherapy used are summarised in Figure 2. (p.39)

Non-specific immunotherapy

(1) Systemic

The majority of immunological stimulants have been avirulent bacteria and fungi or extracts of these. A few have been relatively unknown before and, though claims for benefit have been made, their value is unproven. Examples of these include schizophyllan, a polysaccharide prepared from a mushroom-like fungus (Oshima, Izumi, Kado, Sato and Honda, 1980), bestatin, derived from Streptomyces olivoreticuli (Svanberg, Widell and Cronberg, 1980) and O.K. 432, prepared by incubating haemolytic streptococci with potassium penicillin (Watanabe, Iwa and Yamamoto, 1980). Most studies of non-specific immunotherapy have involved mycobacterial antigens, corynebacteria or immunorestorants such as levamisole and thymosin.

Occasional reports of the use of these in advanced lung cancer still appear. For example Pines (1980) combined repeated percutaneous BCG. with regular oral levamisole in patients with locally inoperable squamous cell carcinoma who had received radiotherapy. In patients receiving levamisole and BCG. twice weekly there was improved survival during the first 2 years only.

Early experience suggested that immunotherapy was likely to be effective only where the tumour cell population had been reduced to a minimum by surgery, radiotherapy

or chemotherapy (Ritts, 1979). Most interest has thus centred round its use in surgical cases. Table 28 summarises some of the better known trials of systemic non-specific immunotherapy. In the majority of these, increased survival compared with controls has been observed. Two exceptions were British studies in whom a relatively low dose of BCG. was given over a short period of time (Edwards and Whitwell, 1978; Millar, Roscoe, Pearce et al., 1981).

(2) Local

Considerable interest was aroused by the early report of a highly significant difference in the incidence of tumour recurrence between patients receiving one post-operative injection of intrapleural BCG. and controls (McKneally, Maver and Kausel, 1976). Significant differences in freedom from tumour recurrence ($p = 0.03$) and survival ($p = 0.04$) were still being reported 4 years later (McKneally, Maver, Bennett and Ruckdeschel, 1980). However these results have not been confirmed in the United Kingdom (Lowe, Iles, Shore et al., 1980, Table 29) or in a large multicentre trial in North America which has yet to be published. It has been suggested that isoniazid, given to controls as well as to the BCG. cases in the original study, might have increased the incidence of tumour recurrence in this "unprotected" group. However this theory was refuted by the finding that the controls had a similar survival curve to that of other surgical cases not included in the study (McKneally, Maver, Alley et al., 1979). Intrapleural C. parvum has been under

Authors	Immunotherapy	Time after operation	Percentage patients surviving		Significance
			Treated	Controls	
Djurovic and Decroix, 1977	Heat-killed myco-bacterial suspension	2 years	80	40	$p < 0.001$
Edwards and Whitwell, 1978	Single subdermal BCG. Glaxo injection	5 years	30	20	N.S.
Pouillart et al. 1979*	Percutaneous BCG. Pasteur	2 years	77	52	$p < 0.05$
Miyazawa et al., 1979	Percutaneous BCG. Japan	2 years	100	69	$p < 0.02$
Millar et al., 1980	Intradermal and percutaneous BCG. Glaxo	5 years (nodes negative)	47	23	N.S.
Amery, 1980	Levamisole	2 years	65	57	N.S.
Hadziev et al., 1980	BCG. or soluble fraction F 70	2 years	47	24	$p = 0.01$

Table 28. Actual or projected (indicated by *) survival after operation of patients treated with non-specific immunotherapy.

Authors	Immunotherapy	Results
McKneally *et al.*, 1979	Intrapleural BCG. Tice	2 year survival of stage I BCG cases = 92%, controls = 60%, $p < 0.01$
Lowe *et al.*, 1980	Intrapleural BCG. Glaxo	2 year survival of stage I BCG. cases = 59%, controls = 66%, N.S.
Ludwig Lung Cancer Study Group, 1980	Intrapleural *C. parvum*	No difference between BCG. group and controls, mean follow-up 60 weeks.
Holmes, 1981	Intralesional BCG.	Disease free survival over median follow-up of 14 months: BCG. group = 55%, controls = 37%.

Table 29. Results of local non-specific immunotherapy in patients who have undergone resection of lung cancer.

investigation by the Ludwig Lung Cancer Study Group (1980) but the early results have not been promising. However this treatment has been shown to be beneficial in patients with malignant pleural effusions (Millar, Hunter and Horne, 1980).

As some of the most impressive observations of the effect of BCG. have been made after intralesional injection of superficial tumours in animals and man, a group from U.C.L.A. have experimented with injection of BCG. into lung tumours (Holmes, Ramming, Mink et al., 1977). This injection has been achieved by percutaneous insertion of a needle into the tumour on one occasion three weeks before thoracotomy in patients with operable lung cancer. Early follow-up results are promising (Holmes, 1981). In tuberculin negative patients, this treatment produced a typical granulomatous inflammatory reaction in the tumour. This reaction was identical to that seen in animal models and associated with systemic anti-tumour immunity. Tuberculin positive patients had a different histological reaction; there was widespread fibrinoid necrosis with an inflammatory reaction consisting of more plasma cells and the occasional formation of germinal centres within the tumour. Of particular interest was the finding that, while lymphocytes of tumours from untreated patients were non-cytotoxic, BCG.-injected tumours contained lymphocytes which were cytotoxic to tumour cells in vitro. Trans-bronchoscopic intralesional injection of BCG. (Glaxo) has also produced significant clinical and radiographic improvement (Millar, Hunter, Wightman and Horne, 1980).

Combination of non-specific immunotherapy with chemotherapy

Many workers, especially Japanese, have sacrificed the chance of assessing the role of immunotherapy alone by using it only as an additive in patients who are also being treated with a complicated chemotherapeutic regime. For example, Watanabe et al. (1980) compared treatment with O.K. 432 plus chemotherapy with chemotherapy alone in 160 patients undergoing surgical resection. Of those having a curative resection, 64% in the immunotherapy group and 52% in the chemotherapy group survived 5 years. This was considered to be a significant difference. In this context, immunotherapy may simply restore cell-mediated immunity depressed by chemotherapy rather than act as an anti-cancer agent in its own right. This reservation also applies to patients in whom immunotherapy is combined with radiotherapy. Van Houtte, Rocmans, Bondue et al. (1979) using levamisole and Kerman and Stefani (1978) using BCG. achieved better clinical results in patients receiving radiotherapy and immunotherapy than in those receiving radiotherapy only.

Specific immunotherapy

Three trials of specific immunotherapy in lung cancer are summarised in Table 30. In each of these, allogeneic tumour cells with a mycobacterial adjuvant were employed. In each study, the treated group fared better than controls although the difference was significant only at the $p = 0.06$ level in the N.C.I. study (Perlin, Oldham, Weese et al., 1980). Clinical results in our own study are given in Tables 21 to 27 and in Figures 28 to 32. They

Authors	Immunotherapy	Clinical measurement	Treated group	Controls	Significance
Perlin et al., 1980	Irradiated allogeneic tumour cells	Percentage survival at 2 years	72	61	$p = 0.06$
Stewart et al., 1980	Soluble allogeneic lung cancer antigen homogenised with Freund's adjuvant $\pm$ methotrexate	Percentage survival at 2 years	100	85	$p < 0.01$
Takita et al., 1981	Pooled allogeneic squamous cell carcinoma with Freund's adjuvant	Projected 3 year survival	89	44	$p < 0.05$

Table 30. Some examples of specific immunotherapy in patients who have undergone resection of lung cancer.

show an advantage for the autograft group when actuarially calculated curves based on survival data during a follow-up period of between two and five years are used. However the difference in survival is significant only in patients with stage I tumours and in those with positive reactions to DNCB. before operation. When freedom from disease recurrence was measured in the same way, a significant difference between autograft and non-autograft patients was seen only in stage I and DNCB. positive cases (Tables 22 and 24). The percentages remaining alive and free from tumour recurrence at 2 years are given in Table 27. Although the autograft group have fared better in both respects, the difference was not significant.

Here are some possible reasons why patients in the autograft group fared better than non-autograft patients:

1. Faulty randomisation

Randomisation was carried out by a lay clerical assistant who did not know the patients or details of their medical condition so that "selection" of envelopes could not have taken place. However the randomisation cards were stratified only for age and sex and not for stage, histology or immunological reactivity. As can be seen from Table 8, the two groups were equally matched except for a relative excess of large cell anaplastic tumours, stage I patients and pneumonectomies in the autograft group. All these factors might be expected to introduce bias against the autograft group. It can be seen that DNCB. reactivity before operation was similar in the two groups.

2. Imbalance in number of cases with T_1N_0 grading within stage I

Shields (1980) drew attention to the importance of TNM classification within stages in the prognosis of surgical lung cancer patients. Tumours are classified as T_1 if they are 3 cm. or less in diameter, surrounded by lung or visceral pleura and without evidence of invasion proximal to a lobar bronchus at bronchoscopy (American Joint Committee for Cancer Staging and End-results Reporting, 1979). T_2 applies to tumours that are more than 3 cm. in diameter, invade visceral pleura or cause atelectasis or pneumonia extending to the hilar region but that are at least 2 cm. distal to the carina at bronchoscopy. T_3 tumours are those of any size which are more proximal than 2 cm. from the main carina and which invade adjoining structures. Nodal involvement is classified into N_0 (no lymph node metastases), N_1 (metastases in peribronchial and/or ipsilateral hilar lymph nodes) and N_2 (metastases in mediastinal lymph nodes). Tumours confined to the chest that are T_1N_0, T_1N_1 and T_2N_0 are classified as Stage I and those that are T_2N_1 as Stage 2. Stage 3 includes any tumour confined to the chest that is T_3 and/or N_2 as well as those with distal metastases (M_1) Shields held that T_1N_1 tumours would be better placed in stage 2 rather than stage I in view of their much worse prognosis than T_1N_0 and T_2N_0 tumours. Among our patients, only one non-autograft patient had a T_1N_1 tumour but there was a larger proportion

of T_1N_0 tumours in the autograft Stage I tumours (13/24) compared with the non-autograft stage I tumours (9/30). Although Shields (1980) found a difference in survival between patients with T_1N_0 tumours and patients with T_2N_0 tumours, patients with T_1N_0 tumours in our series fared rather worse than the whole group of stage I patients (Table 31).

3. Tumour enhancement by preoperative BCG. in non-autograft group

In a previous review, Bast, Zbar, Borsos and Rapp (1974) described criteria necessary for successful BCG. immunotherapy of tumours. One requirement was that there should be an adequate number of organisms injected relative to the number of tumour cells. It has been suggested that inadequate numbers of BCG. organisms could lead to tumour enhancement. It is thus important to consider whether the single preoperative vaccination with BCG. could have worsened the prognosis of the non-autograft group. This can be done by comparing the clinical results for this group with those taken from other surgical series (Table 32).

It can be seen that the 2 year survival results for all the non-autograft patients and for those with stage I tumours are similar to the previous results from Glasgow (Reid, Stevenson, Welsh and Barclay, 1961) and Edinburgh (Le Roux, 1968) though worse than those reported from two centres in U.S.A. Reid et al. (1961) blamed their relatively poor results on concomitant chronic bronchitis

Stage	Autograft		Non-autograft	
	No.	%	No.	%
I (all cases)	17	71	13	43
T_1N_0	8	62	1	11

Table 31. Patients with stage I and the T_1N_0 subgroup tumours who were alive and free from tumour recurrence 2 years after operation.

Authors	Histology	All Patients	Lobectomy Patients	Pneumonectomy Patients
West of Scotland Lung Cancer Group, 1981	Non-small cell (control group)	36	42	31
Le Roux, 1968, Edinburgh	All types	-	47	24
Reid et al., 1961, Glasgow	All types	32	-	-
Mountain et al., 1980, Houston	Squamous cell	45	-	-
Shields et al., 1980, Chicago	Squamous cell	47	-	-

Table 32. Percentage of patients surviving 2 years after operation showing that results for the control group are similar to those of two Scottish reports.

and emphysema which is so prevalent in Scotland. An additional factor is undoubtedly the high prevalence of coronary artery disease. 4 of our 83 patients died from myocardial infarction more than one year after recovery from their operation.

4. Effect of specific immunotherapy

The fourth reason for the difference may be that autografts of irradiated cells with adjuvant BCG. do increase the rejection of residual tumour cells by the defence mechanism. Some support for this view comes from the finding that patients capable of mounting a cell-mediated reaction to a foreign hapten DNCB. seem most able to benefit from the specific immunotherapy given. A second point is that the time at which the clinical effect is most obvious (12 months) comes towards the end of the period when tuberculin reactivity of the autograft group is significantly raised above base line. The results show clearly that the beneficial effect is only temporary and this has clear implications for the design of future trials.

It has been suggested that, if percutaneous or intra-dermal immunotherapy increases survival, it may do so by stimulating the rejection of tumour cells which have been deposited in distant organs. Robinson et al. (1977) found that administration of methanol extraction residue (MER.) of BCG. to lung cancer patients having radiotherapy and/or chemotherapy reduced the incidence of visceral metastases. A sign of effective immunotherapy might

therefore be a relative fall in the incidence of distal metastases compared to that of local recurrence. However in our study, the pattern of recurrence was similar in both the autograft and non-autograft group (Table 33).

Unwanted effects

(a) Local

Moderately severe local ulceration can occur when BCG. and related mycobacterial antigens are injected into the skin. Perlin et al. (1980) had to reduce the number and frequency of Heaf gun BCG. punctures in 43% of their cases. Particularly severe local reactions occurred in the autograft group of the pilot trial of this study when BCG. was injected intradermally (Figure 9). These occurred especially in strong tuberculin reactors. Such reactions are more severe when BCG. is given on the same day as levamisole (Pines, 1980).

Local reactions also occur when BCG. is given intrapleurally in postoperative patients (Law, Spiro, Geddes and Hodson, 1981). Eleven out of 39 of their cases developed these; empyema in 6 and severe wound infection in 5. Similar complications were reported by McKneally et al. (1976a). Though previously reported after intradermal injection of melanoma cells, local tumour growth has not been reported after injection of lung cancer cells.

	Local	Distant
Autograft group	7	8
Non-autograft group	10	10

Table 33. Pattern of tumour recurrence showing similar distribution in both groups.

(b) Systemic

Non-specific febrile reactions have occurred between 12 and 48 hours after cutaneous application of BCG. in about half of the patients so treated. Transient elevation of serum transaminases has occurred after intrapleural BCG. (McKneally et al., 1976a) and C. parvum (Fox, Woods, Tattersall and Basten, 1980). Severe hypotension occurred in one of the patients treated with C. parvum. On the whole lung cancer patients have tolerated immunotherapy well and even intralesional BCG. has not so far produced disseminated BCG. infection.

(c) Tumour enhancement

A clear warning that immunotherapy can enhance tumour growth has come from numerous animal experiments (e.g. Colmerauer, Koziol and Pilch, 1980). Possible examples of enhancement of lung cancer have come from Nilsson and Afeldt (1975) and McCracken, Heilbrun, White et al. (1980).

Defects of immunotherapy trials

It may surprise scientists working in other fields that even now, 16 years after the start of modern studies of immunotherapy in lung cancer, the value of immunological treatment is not yet known. While it is clear that immunotherapy is a relatively weak anticancer agent, another factor is undoubtedly the poor design of many trials.

The single most important flaw in many of these is the failure to use comparable controls. In a trial of cancer therapy, the controls must be randomly selected and prospective. Yet historical controls were used in

several important trials including those of Yasumoto et al. (1979) and Hadziev et al. (1980). Even in the study of Jansen, The and Orie (1980) of BCG. immunotherapy in 54 patients with locally advanced squamous cell carcinoma, no controls were added after the first 20 patients in each group had accrued. Moreover patients who refused intrapleural BCG. were included in the alternative systemic immunotherapy arm. It is well known that patients may refuse treatment because their condition is better or worse than more compliant patients. Such selection may prejudice the results of survival studies.

Some workers have reported on the same immunotherapy regime given to a heterogeneous collection of patients in such a way as to make it impossible to separate the effect of immunotherapy from that of the natural course of the illness. For example Yasumoto et al. (1979) administered BCG. cell-wall skeleton (CWS.) to five groups of patients each differing from each other in the site and extent of the tumour.

Another fault is to use too many treatment arms (e.g. McCracken et al., 1980). The result is that each arm contains only a few patients which makes it difficult to draw valid conclusions about differences when they arise. Frequently combined with this defect is the combination of one or more forms of immunotherapy with chemotherapy and/or radiotherapy. If it is possible to detect a significant difference between the treatment arms, one cannot be certain whether it is the immunotherapy alone

or immunotherapy acting synergistically with chemotherapy or radiotherapy that has caused the difference.

DESIGN OF FUTURE TRIALS OF IMMUNOTHERAPY

Our results suggest that specific autologous immunotherapy may delay tumour recurrence in patients with stage I lung cancer especially those who are capable of mounting an immunological response to antigenic material. This trial started in 1975 when relatively little was known about tumour immunotherapy. These results and numerous reports from the literature suggest that a further trial starting this year should have the following modifications:

Timing, duration and nature of immunotherapy

The aim of treatment is to stimulate the immunological system to destroy small populations of tumour cells that are disseminated before, during or just after operation or that are left behind at the operation site. The time of maximum danger is the operation itself when tumour cells may be discharged into the blood (Kuper and Bignall, 1966) and one to two weeks after operation when immunological function is depressed. It thus seems that immunotherapy should be started before operation. Although both autograft and control patients in this study were "primed" with BCG. before operation, the only current trial of preoperative immunotherapy is that of intralesional BCG. by Holmes (1981).

Other studies have shown promising clinical results at 1 and 2 years after operation but no effect of immunotherapy on survival and freedom from tumour recurrence

thereafter (e.g. Edwards and Whitwell, 1974; Pouillart, Palangie and Huguenin, 1979). Although immunotherapy was given for 18 months by Pouillart et al. (1979), in many studies immunotherapy was given only during the immediate postoperative period. In our study the duration of immunotherapy was limited by the volume of autologous tumour cells available. In the intrapleural studies, surgical considerations suggest that repeated injections at the site of the operation might produce more complications than those already encountered (Law et al., 1981). A future trial should therefore consist of immunotherapy prolonged over one or 2 years after operation to reduce the risks of late relapse.

These two considerations rule out the use of autologous tumour cells. This treatment has the theoretical advantage that these cells are not destroyed by ordinary transplantation rejection mechanisms and so may have more time and opportunity to evoke an immunological attack on tumour cells left behind at operation. On the other hand, allogeneic cells possessing different tumour-associated antigens may evoke more immunological reaction in general. With modern methods of storage, these cells can be obtained in relatively unlimited supplies in large centres so that injections of cells or extracts could be given before and at regular intervals after operation over a couple of years. The cells should be rendered more immunogenic with the appropriate enzymes (e.g. neuraminidase). Despite claims for C. parvum, BCG.

is more widely available and there is more evidence of its effectiveness as an adjuvant in patients (Mathé et al., 1969b). By percutaneous administration it can be given repeatedly without undue side-effects although the dose may need to be modified in tuberculin sensitive patients.

Route of immunotherapy

The value of local treatment is still unproven. In the study of McKneally, Maver, Kellar and Lininger (1978) intrapleural BCG. considerably reduced the incidence of local recurrence. However relapse after successful resection of lung cancer is more often due to distal metastases (e.g. in 70% of relapsed cases, Bourgeon, Richelm, Lelan et al. (1980)). This fact and the inability to give local immunotherapy repeatedly over a period of time indicate that a further trial now would still involve intradermal or percutaneous administration of the immunological stimulant. However this could be combined with a single local treatment as in some current trials (e.g. McKneally, Maver, Bennett and Ruckdeschel, 1980).

Staging procedures

(a) Preoperative

Since the start of the original trial, more accurate preoperative staging procedures have become widely available. Liver, bone and CAT. scans should be done to exclude patients with distal metastases and, in the case of CAT. scans, to define more accurately the extent of intrathoracic disease. Mediastinoscopy would be used only in patients where there were doubts over the extent of mediastinal lymph node involvement.

(b) Survival and pathological

Careful assessment of the surgical stage has always been the practice in the units involved in this study. Nevertheless it may be that dissection out of all known groups of mediastinal lymph nodes should be attempted in future. This might improve the clinical results as well as leading to more accurate staging. The operation would be more prolonged but there is no evidence that such dissection causes increased morbidity or mortality.

Randomisation

In this study stratification of patients was carried out only for age and sex. Future studies should stratify for:

(1) Tumour histology

(2) Tumour stage

(3) Immunological reactivity

It seems likely that some of the discrepancy in results between different groups of workers and the marginal benefits of immunotherapy described by some workers is due to differences between samples of patients concealed within broader divisions such as non-squamous cell carcinoma and stage I tumours. It may be that stratification according to TNM. subdivisions of stage I to III should be included.

There is some evidence that certain groups of patients are more likely to respond to immunotherapy, e.g. tuberculin positive patients (Jansen et al., 1980) DNCB. positive patients (Chen, Ogino, Wada, Matsumoto et al., 1980) and those with an acceptable level of active T rosette forming

lymphocytes (Kerman and Stefani, 1978). In future trials of immunotherapy, stratification might take account of some criterion of immunological reactivity measured before operation.

The corollary of such multifactor stratification is that series of patients studied should be large (several hundreds) in order that statistically significant benefits of treatment could be recognised. This implies the need for multicentre trials such as those already in progress in North America and Europe.

Immunological measurements

One of the weaknesses of most published investigations of immunotherapy has been their failure to correlate clinical results with immunological measurements. More success in this field may be achieved with the development of better techniques of measuring macrophage function. It will also be important to assess the *in vitro* effect of the patients' lymphocytes and macrophages on their own tumour cells. The implications for organisers of such projects is that more funding is needed to provide adequate numbers of technicians and the necessary laboratory equipment.

CHAPTER 9

CIRCULATING ANTIGENIC MARKERS AND IMMUNOREACTIVE HORMONES IN LUNG CANCER

The previous sections have dealt with immunological methods of treatment of lung cancer. This section deals with the immunological measurement of circulating tumour markers in the diagnosis and discusses their present place in assessing the prognosis and response to treatment. A classification of these markers is given in Table 34.

SERUM ANTIGENIC MARKERS

One of the most interesting discoveries in the field of cancer diagnosis has been the finding that certain antigenic substances found in foetal and embryonic tissues are also present in high concentrations in malignant tumours. In 1963 Abelev, Perova, Khramkova et al. described the isolation of α-foetoprotein (AFP.). Two years later came the first description of carcinoembryonic antigen (CEA.) by Gold and Freedman (1965). CEA. is a glycoprotein, MW. approximately 200,000 daltons which elutes between IgG and IgM on a Sephadex-G 200 column (Terry, Henkart, Coligan and Todd, 1974). Thomson, Krupey, Freedman and Gold (1969) developed a radioimmunoassay for this and showed that 35/36 patients with large bowel tumours had detectable levels in the serum. Subsequently measurement of CEA. has become established as a useful test in detecting recurrence of bowel cancer following surgery.

Most estimations of oncofoetal antigens involve radioimmunoassay. In the case of CEA., anti-CEA. antiserum raised in animals is added to the test serum together with a fixed quantity of radioisotopically-

1. Oncofoetal

    Carcinoembryonic antigen

    α-foetoprotein

2. Placental

    Human chorionic gonadotrophin and β subunit

    Human placental lactogen

3. Ectopic hormones

    Pro-ACTH

    Calcitonin

    Antidiuretic hormone

    Parathormone

    β-lipotrophin

4. Others

    Ferritin

    Lactoferrin

    Casein

    β_2-microglobulin

    Caeruloplasmin

    Pregnancy-associated α_2-glycoprotein

Table 34. Antigenic markers in lung cancer.

labelled CEA.. Unlabelled CEA. in the patient's serum competes with the labelled CEA for the antibody binding sites. Antibody-bound CEA. is precipitated chemically using zirconyl phosphate (Hansen, Lance and Krupey, 1971) or with an anti-immunoglobulin (double antibody technique) (Egan, Lautenschleger, Coligan and Todd, 1972). Comparison of the percentage of labelled CEA. bound in the test serum with the percentage bound in the presence of known amounts of CEA. allows quantitation of CEA. in the sample.

CEA. levels have now been measured in several series of patients with lung cancer (Table 35). The two main types of radioimmunoassay used are:

1. the perchloric acid technique (Hoffman La Roche test) (Hansen et al., 1971).
2. the direct radioimmunoassay method (Egan et al., 1972). In this higher values are obtained depending on whether they are referred to serum-based standards.

Diagnostic value of antigenic markers

The disadvantage of CEA. as a reliable diagnostic screening test in lung cancer is that elevated levels have never been found in more than 75% of unselected cases (Table 35). In patients with resectable tumours the incidence of raised CEA. levels was much less e.g. 14% (Ford, Newman and Anderson, 1979) and 18% (Concannon, Dalbow, Hodgson et al., 1978b). Previous reports have shown elevated levels of pregnancy associated α2 glycoprotein (α_2-PAG.) in 60% (males only, Gropp, Lehmann, Bauer and Havemann, 1977), the β subunit of human chorionic gonadotrophin (β-HCG.) in 16% and K-casein in 34% (Reddy,

Authors	Method	Reference values (ng/ml)		Patients	Prevalence of raised CEA.%	
		Normal	Smokers or benign disease		> Normal	Smokers or > benign disease
Ford et al, 1977	Direct RIA.	-	50	256, mainly operable	-	6
Bisset et al, 1978	Direct RIA.	10	40	88 unselected	64	21
Broder, 1980	Perchloric acid extraction	-	5	126 unselected	-	55
Gropp et al., 1979a	Direct RIA.	10	-	171 unselected	51	-
Vincent et al., 1975	Perchloric acid extraction	2.5	8.7	228 unselected	68	-
Paone et al., 1980	Automated indirect RIA.	10	16	180 unselected	73	48
Waalkes et al., 1980	Perchloric acid extraction	2.5	5	42 small cell carcinoma patients	74	48

Table 35. Incidence of raised serum CEA. levels in larger series of lung cancer.

Rochman, Hunter et al., 1979). While this rate of positive tests is far too low to allow the use of individual markers as diagnostic screening tests, combination of tests might produce a more accurate method of diagnosis. For this reason, we decided to measure five antigenic markers in recently diagnosed lung cancer cases and to assess the value of combined measurements in diagnosis.

METHODS

Patients

197 patients were studied in three Glasgow hospitals. Samples of blood were taken from the patient on first attendance at the chest clinic or shortly after admission to the ward. It will be noted that even at this relatively early stage of investigation nearly half the patients had clinical or radiographic evidence of metastases.

All the controls were patients attending a chest clinic or admitted to the ward for investigation or follow-up of non-malignant pulmonary or cardiac disease. 27 out of 70 (39%) suffered from chronic bronchitis, asthma and/or emphysema and 21 (30%) from healed or active pulmonary tuberculosis. The ages and sex of the lung cancer patients and controls were similar (Table 36). None of the patients or controls were taking cortico-steroids or other immunosuppressive drugs. Although the smoking habits of the controls were not recorded, they are likely to have been similar to those of the lung cancer patients in view of the high proportion of patients with chronic obstructive airways disease and of our findings with a similar control population in the ACTH. study (q.v.).

		Lung Cancer	Controls
Males:	Number	158	52
	Mean age (years)	63.4	60.7
	Range of ages (years)	26-85	42-86
Females:	Number	39	18
	Mean Age (years)	61.3	59.4
	Range of ages (years)	45-83	46-71

Table 36. Age and sex of lung cancer patients and controls.

Laboratory Methods

Serum was separated from the blood samples and was stored at -20°C until the relevant measurements could be made. The laboratory methods used are detailed in Table 37.

RESULTS

Only 3 of 70 control patients had CEA. levels greater than 40 ng/ml and none had levels greater than 100 ng/ml. In contrast, 34 of the 197 lung cancer patients had elevated levels and 23 had levels in excess of 100 ng/ml. The mean values for CEA. were significantly higher in the lung cancer group compared with controls (Table 38).

As the majority of lung cancer patients in the West of Scotland smoke cigarettes, suffer from chronic bronchitis and/or have had pulmonary tuberculosis, we have calculated the number of lung cancer patients with antigenic marker levels greater than the upper 95th percentile of the controls. In this way we hoped to estimate the extent to which elevated marker levels could be attributed solely to the tumour.

Separate levels for pregnancy-associated α_2-glycoprotein (α_2-PAG.) were calculated in males and females because of the difference in normal values of this marker between the sexes. Because many patients had serum concentrations below the minimum detectable levels, mean values for markers other than CEA. could not be calculated.

The overall prevalence of elevated levels of individual markers never exceeded 20% although the

Antigenic marker	Method	Authors
CEA.	Double antibody direct radioimmunoassay with unextracted serum	Egan et al., 1972
α_2-PAG.	Sandwich immunoassay using horse radish peroxidase as marker for antibody to α_2-PAG.	Stimson and Sinclair, 1974
Casein	Double antibody radioimmunoassay with unextracted serum	Hendrick and Franchimont, 1974
AFP.	Polyethylene glycol	Vince, McManus, Ferguson-Smith and Ratcliffe, 1975
HCG.	Double antibody radioimmunoassay using unextracted serum	Vaitukaitis, Braunstein and Ross, 1972

Table 37. Immunoassay methods used to measure serum antigenic markers.

	Controls	Cancer
Number	70	197
Range of CEA. ng/ml	11.2 - 66.0	5.0 - 720.0
Mean CEA. ng/ml	24.2	52.6
Number with CEA. > 40 ng/ml	3(4%)	34(17%)

Table 38. Serum CEA. levels in lung cancer patients and controls.

prevalence was higher than 20% in certain subgroups where the total number of cases was small (Table 39, from Burt, Ratcliffe, Stack et al., 1978). 38% of controls, 50% of patients with localised disease and 49% of patients with metastases had undetectable levels of AFP. in the serum. In no control patients could HCG. be detected and measurable levels were found in only 2/58 patients with localised disease and 5/55 with metastases.

When the results for different markers were combined, some concordance of elevated HCG., AFP., and casein levels was found. Thus of 7 patients with elevated HCG. levels, one also had elevated CEA.; of 6 with elevated casein, 2 had elevated CEA; and of 2 with elevated AFP., one had elevated CEA..

In contrast there was a negative correlation of CEA. and α_2-PAG. (r -0.22, $p < 0.05$, $n = 89$). All patients with elevated CEA. levels had normal α_2-PAG levels and vice versa (Table 40).

The mean CEA. level was significantly higher in patients with metastases than in those with localised disease (Table 41). Similarly the prevalence of a raised CEA. level was greater in patients with metastatic disease. This pattern was not confirmed for other markers (Table 39).

Figure 34 shows the CEA. levels in the individual patients with a histological diagnosis. There is no clear relationship between histology and CEA. concentration. However this series contained only 2 patients with adeno-carcinoma, the tumour in which the highest proportion of

Antigenic marker		Upper 95th centile of controls	Prevalence of elevated levels		
			Localised	Metastatic	All cases
CEA.		40 µg/l	12/101 (12%)	22/93 (23%)	34/194 (17%)
α_2-PAG.	Males	70 µg/l	6/36 (17%)	5/39 (13%)	15/92 (16%)
	Females	130 µg/l	3/11 (27%)	1/6 (17%)	
Casein		25 µg/l	3/24 (15%)	3/18 (17%)	6/42 (14%)
β-HCG.		2 µg/l	3/56 (5%)	4/56 (7%)	7/112 (6%)
AFP.		10 µg/l	0/62 (0%)	2/72 (3%)	2/134 (1.5%)

Table 39. Prevalence of elevated levels of antigenic markers in lung cancer.

Elevated CEA. levels		Elevated α_2-PAG levels	
CEA. (µg/1)	α_2-PAG. (mg/1)	CEA. (µg/1)	α_2-PAG. (mg/1)
240	0.2	19.4	86
43	0.2	29.3	155
395	26.0	18.0	128
238	25.0	10.8	133
195	0.2	24.0	96
193	9.0	30.0	108
140	57.0	16.7	135
520	12.0	15.2	250
55	0.2	24.0	105
220	0.2	14.8	127
135	0.2	38.0	166
340	0.2	27.8	131
45	50.0	10.8	72
42	22.0	15.0	118
195	20.0	20.0	80
42	18.0		

Table 40. Discordance between elevated CEA. and α_2-PAG. levels in patients with lung cancer.

	Localised	Metastatic
Number	101	93
Mean CEA. (ng/ml)	32	74
Range of CEA. (ng/ml)	5 - 280	8 - 720
Number 40>ng/ml	12 (12%)	22 (23%)

Table 41. CEA values in patients with localised and metastatic disease.

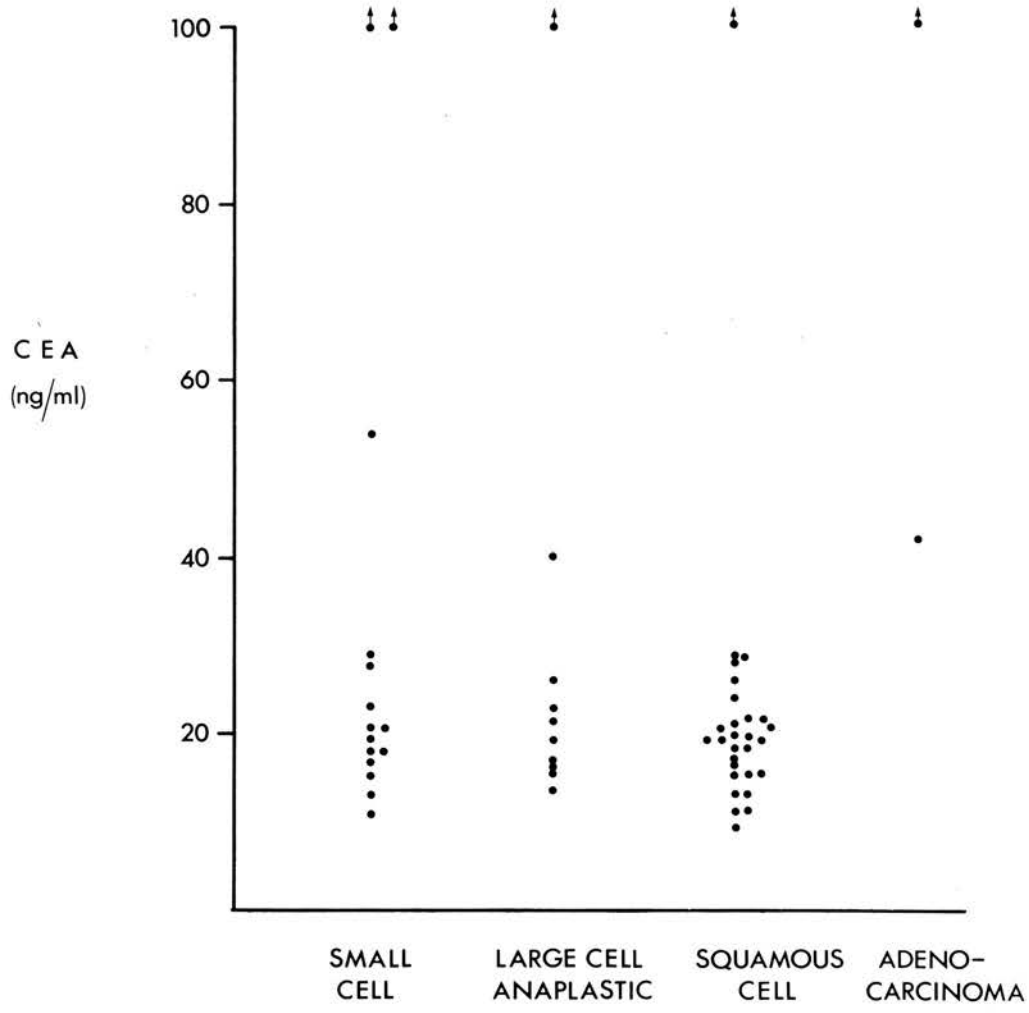


Fig. 34 Serum CEA values in unselected lung cancer patients according to histology.

elevated CEA. levels have been reported (Ford, Newman and Lakin, 1977; Bisset, Roux, Sauvan et al., 1978).

DISCUSSION

Value of antigenic markers in diagnosis

CEA. is the principal serum antigenic marker measured in lung cancer. In the majority of reports, elevated levels have been found in more than half of the patients studied when these have been compared with normal controls (Table 35). Our results show a much lower prevalence of raised serum CEA. and are closer to those of Ford et al. (1977) and Bisset et al. (1978). This discrepancy is not due to the methods used. The perchloric acid extraction method (Hansen et al., 1971) was used by three American groups (Vincent, Chu, Fergen and Ostrander, 1975; Broder, 1980; Waalkes, Abeloff, Woo et al., 1980) whereas others, including all the European groups, used a double antibody direct radioimmunoassay (RIA.). Values of 2.5 ng/ml and 5 ng/ml in the former are roughly the equivalent of values of 20 and 40 ng/ml in the latter. It can be seen that a similar prevalence of above normal CEA. values was obtained by workers using the perchloric acid extraction method (Vincent et al., 1975; Waalkes et al., 1980), those using the direct RIA. method (Gropp, Lehmann and Havemann, 1979a) and those using an automated indirect RIA. (Paone, Kardana, Rogers et al., 1980).

A major factor in this discrepancy is undoubtedly the selection of the reference value. Vincent et al. (1975) derived this from levels in normal blood donors.

These might be expected to be healthier than a population of lung cancer patients and to have a lower prevalence of cigarette smoking. Elevated levels of CEA. are commoner in smokers (Stevens, Mackay and Busselton Population Studies Group, 1973) and in patients with benign pulmonary disease (Hansen, Snyder, Miller et al., 1974). Lung cancer patients are usually smokers (81% in the immuno-reactive hormone study, see below) and frequently also suffer from chronic bronchitis. Hence it is more logical to use smokers with benign pulmonary disease as controls. In Table 35, it can be seen that the prevalence of elevated CEA. levels was much reduced in three series when the reference value was derived from smokers and/or patients with benign diseases. Had we taken 20 ng/ml as the upper limit of normal, as did Grigor, Detre, Laurence et al. (1975), 64% of our patients would have had elevated levels of CEA..

Even taking the series with the highest percentage of CEA. levels above those of smokers or patients with benign pulmonary (55%, Broder, 1980) it is clear that serum CEA. estimation falls well short of the requirements for a diagnostic or screening test.

CEA. has also been estimated in bronchial secretions aspirated at bronchoscopy (Aubanel, Milano, Schneider et al., 1978). However the prevalence of raised levels was much higher in patients with bronchoscopically visible and therefore already diagnosed tumours than in those with more peripheral lesions. Moreover raised levels of

CEA. also occurred in benign pulmonary inflammatory conditions. Despite the fact that serum and tissue levels of CEA. are usually highest in adenocarcinomas (Ford et al., 1977; Sun, Bennett, Carpentier and Terry, 1976), of which a higher proportion are peripheral tumours, measurement of bronchial aspirate CEA. was of little help in diagnosis. The value of measuring pleural fluid CEA. was assessed by Thomson, Rana and Ratcliffe (1979). From their own results and those of five other groups they concluded that it was a useful addition to pleural biopsy and fluid cytology with a test sensitivity around 40%.

As the prevalence of raised serum levels of single markers was too low for them to be individually useful in the diagnosis of lung cancer, the value of combining measurements of several markers has been considered. A surprising finding has been the negative correlation of CEA. with α_2-PAG., a high MW. glycoprotein found especially in pregnancy but also found in patients of both sexes with breast and lung cancers (Stimson, 1975). This negative correlation was not due to α_2-PAG. acting as a carrier for CEA. (Burt et al., 1978) and no explanation for it has yet been discovered. One or more of the 5 markers was elevated in 46% of those patients who had 4 or more markers measured. This contrasts with elevation of one or more of CEA., β-HCG. and casein in 76% of patients studied by Reddy et al. (1979). However both results fall short of the prevalence necessary to make even this combination of tests useful in the diagnosis of lung cancer.

AFP. is not a useful tumour marker for lung cancer having been found in the serum of none of the patients of Gropp et al. (1977) and in only 3% by Grigor et al., (1975). Similarly as other workers have found elevated levels of HCG. in only 34% (Gropp et al., 1980), 16% (Reddy et al., 1979) and 12% (Broder, 1980), our finding of raised levels in only 6% confirms the uselessness of this antigenic marker in the diagnosis of lung cancer.

Serum casein is a normal protein constituent of milk. Initial attempts to measure blood levels were made in pregnant and lactating women. The finding of significant levels in all lactating women led to the investigation of this protein in patients with breast cancer in whom 1 in 7 had detectable levels. This led to speculation as to whether the substance could be produced by non-endocrine tumours and significant levels were found in all lung cancer patients tested (Hendrick and Franchimont, 1974). 42 of the 197 patients in our series were tested for this antigen but the levels were significantly raised in only 14%. Thus while further evaluation using more sensitive assay techniques would be worth considering, at present serum casein is not likely to be of major value in the diagnosis of lung cancer.

Prognostic Value of Antigenic Markers

Serum antigenic markers may be of value in assessing:

1. The stage and resectability of tumours
2. The response to treatment
3. The recurrence and progression of tumours.

Radical resection of lung cancer is undertaken in patients believed preoperatively to have Stage I and Stage 2 tumours. Most surgeons do not operate on patients in whom clinical and radiographic investigations have suggested that their tumour is Stage 3. It would thus be helpful if measurement of CEA. discriminated between Stages I and 2 and Stage 3. Although Dent, McCulloch, Wesley-James et al. (1978) did find a mean CEA. level of 7.3 ng/ml in inoperable cases compared with 3.1 ng/ml in operable cases, the incidence of elevated CEA. levels in Stage I and Stage 2 (18%) and Stage 3 (25%) was not significantly different in another study (Concannon et al., 1978b). However a very considerably raised CEA. in an apparently operable case does suggest a poor prognosis. Only two patients with CEA. greater than 10 ng/ml (upper limit of "normal" 2.5 ng/ml) in the series of Vincent, Chu and Lane (1979) survived their operation by more than two years. Similarly Ford et al. (1979) found that 11 of 14 patients with pre-operative levels greater than 40 ng/ml developed metastases or had locally ineradicable tumour.

In a larger non-surgical series, Gropp, Havemann and Scheuer (1980) found CEA. levels greater than 10 ng/ml (upper limit of "normal") in only 16% of patients with disease confined to one hemithorax and the ipsilateral cervical nodes but in 82% of patients with more disseminated disease.

Serum levels of other markers have also been of only

limited value in assessing the stage of the disease. Thus K-casein and β-HCG. did not help to distinguish between T_2 and T_3 tumours, N_1 and N_2 lymph node involvement, or patients with and without distant metastases (Reddy et al., 1979). On the other hand, measurements of serum haptoglobin, orosomucoid and C-reactive protein correlated with tumour size (Bradwell, Burnett, Newman and Ford, 1979) though tumour size was not related to prognosis. However it was found that patients in whom these serum factors were higher than the mean level for their size of tumour had a poor prognosis.

Circulating levels of antigenic markers can also reflect the effects of treatment. In patients with elevated levels of CEA. before operation, the concentration drops to normal levels within five days of tumour resection (Vincent et al., 1979) and after chemotherapy and radiotherapy (Gropp et al., 1979a).

In patients in whom CEA. level does not fall after radiotherapy, tumour progression is likely to be occurring. Moreover elevation of previously normal CEA. may precede clinical and radiographic evidence of tumour recurrence (Gropp et al., 1980).

In view of the apparent relationship between antigenic markers and tumour size (Bradwell et al., 1979) and the evidence that immunotherapy is likely to be effective only where the tumour cell population is small, one would expect antigenic marker levels to give some guide to the likely response to immunotherapy. This has been

confirmed by Gautier, Baron, Huguenin et al. (1978). They found that in 19 of 27 patients with inoperable squamous cell carcinoma who had normal CEA. levels, there was evidence of tumour response to percutaneous BCG. given three times a week for three weeks compared with a response in only 1 out of 12 patients with elevated CEA. levels.

Circulating immunoreactive hormones in lung cancer

Other potential immunological markers for lung cancer are the immunoreactive hormone ACTH. and related peptides.

In 1928 Brown described the occurrence of thirst, anorexia and weakness in a 45 year old woman. She was found to be pigmented and to have other clinical features of Cushing's Syndrome including glycosuria. Her condition deteriorated and at autopsy she was found to have a small cell carcinoma of the lung.

The occurrence of a Cushing-like Syndrome in association with lung cancer subsequently became recognised as an occasional non-metastatic complication. Estimates of its incidence in unselected series of cases have included 0.5 to 2% (Yesner, 1978), 0.5% (Azzopardi, Freeman and Poole, 1970) and 0.4% (Rassam and Anderson, 1975). The vast majority of these cases have occurred in association with small cell carcinoma.

That this syndrome was due to excessive ACTH. production by the tumour was eventually established by Meador, Liddle, Island et al. (1962). They found elevated concentrations of ACTH. in the tumour tissue and in the

plasma of five patients, three of whom had small cell carcinoma of the lung. The urinary 17-hydroxycorticosteroids were elevated and dexamethasone did not reduce their concentration.

Meador et al. (1962) used a bioassay of ACTH. but nearly all the work in the past 12 years has been based on immunological methods. One of the earliest descriptions of this approach was that of Berson and Yalow (1968). In their radioimmunoassay, the isotopically labelled peptide under investigation (e.g. ACTH.) is bound to a specific antibody raised in experimental animals (e.g. guinea pigs). Competitive inhibition of the binding of this specific antibody is measured when the plasma or serum under test is added. This competitive inhibition is compared with that occurring when standard solutions of ACTH. are added. The ratio of bound to free ACTH. is calculated for the test plasma or serum and the absolute ACTH. value is read off from curves prepared using the standard solutions.

Berson and Yalow found that ACTH. levels in plasma were lower in the evening. They were depressed by dexamethasone and increased by hypoglycaemia, shock and surgery under general anaesthesia.

Armed with a comparatively simple assay method, the same group of workers measured levels of ACTH. in pituitary gland, tumour tissue and plasma. An early discovery was that the nature of the ACTH. varied (Yalow and Berson, 1971). The predominant form in tumour tissue and the plasma of tumour patients was not the normal pituitary

1-39 ACTH. but a large molecular weight more acidic peptide. Referred to as "big ACTH", this had a molecular weight of approximately 20,000 daltons (Wolfsen and Odell, 1979). On filtration through Sephadex G50 columns, its elution volume lay between human growth hormone M.W. 20,000 and serum albumen M.W. 68,000 (Yalow and Berson, 1973). Big ACTH was rapidly broken down to 1-39 ACTH. on exposure to trypsin (Gewirtz, Schneider, Krieger and Yalow, 1974a). Its immunoreactivity was indistinguishable from that of 1-39 ACTH.. However, while the biological and immunological reactivities of the latter were similar, the biological activity of big ACTH. was less than 4% of its immunological activity. This was shown by measuring its ability to stimulate corticosterone production by the adrenal glands of Sprague-Dawley rats.

One result of this low biological activity is that ectopic big ACTH. has relatively little effect on the adrenal cortex. Plasma cortisol was not higher in lung cancer patients with elevated ACTH. levels than in lung cancer patients with normal ACTH. levels (Gropp, Havemann, Scheuer and Grun, 1979b). Consequently feedback suppression of pituitary ACTH. production would not be expected. Thus the overall effect of ectopic ACTH. production by tumour is an increase in total circulating immunoreactive ACTH..

The predominance of "big ACTH." in tumour tissue and the circulation of patients with lung cancer suggests that, while neoplastic cells have the ability to manufacture the necessary amino acid sequences to form ACTH., they

lack the enzymes which can trim the precursor large molecule into 1-39 ACTH. (Yesner, 1978).

Circulating ACTH levels

The finding of elevated plasma ACTH. in a large proportion of cases (Ayvazian, Schneider, Gewirtz and Yalow, 1975) raised the prospect of using this measurement in the early detection of lung cancer. Since then a number of other workers have measured circulating ACTH. levels in a variety of lung cancer patients and the results are summarised in Table 42. All groups have used radio-immunoassay techniques based on that already described (Berson and Yalow, 1968). With this method, ACTH. is detectable at a level 10-20 ng/l (normal range 10-80 ng/l). Thus most workers have expressed their results in terms of the prevalence of plasma or serum concentrations above a given value. In some instances this absolute value has been fixed arbitrarily. In others it has been determined in relation to values for healthy laboratory controls (Hansen, Hansen, Hirsch et al., 1980) or normal subjects and patients with minor illnesses (Wolfsen et al., 1979). This absolute value has varied from 76 to 150 ng/l but as the methods of radioimmunoassay used vary between laboratories, the variation in absolute value is not of great significance.

The reports from the Mount Sinai Hospital, New York (Gewirtz and Yalow, 1974; Ayvazian et al., 1975) showed that ACTH. levels above the arbitrary upper limit of normal were recorded in about one third of patients with chronic obstructive pulmonary disease. The same group had

Authors	Patients	Sample and method	Reference value (ng/l)	Derivation of reference value	Percentage prevalence of patients with raised values		
					Small cell	Non-small cell	All patients
Gropp et al., 1980	unselected	plasma and serum un-extracted	80	age and sex-matched normal controls	30	10	19
Yalow et al., 1979	surgical, non-small cell	plasma un-extracted	150	normal control range	-	72	72
Ayvazian et al. 1975	unselected	plasma un-extracted	150	normal control range but 6% "normals" above	100	72	88
Hansen et al., 1980	unselected, small cell	plasma un-extracted	76	mean + 2 S.D. of laboratory controls	29	-	29
Wolfsen and Odell, 1979	unselected, radiographic abnormality	plasma extracted	107	mean + 2 S.D. of normals and patients with minor illness	-	-	74
Torstensson et al., 1980	unselected	plasma un extracted	175	mean + 1 S.D. other pulmonary diseases	-	-	27

Table 42. Prevalence of elevated ACTH. concentration in plasma and serum of lung cancer patients before treatment.

also found immunoreactive ACTH. in lung tissue from smoking dogs with atypical epithelial changes. Moreover Yalow (1979a) reported that two patients from this group who subsequently died had precancerous epithelial changes at autopsy but no evidence of lung cancer. As the population at risk of developing lung cancer consists of male smokers over 40, many of whom have already developed chronic bronchitis, the value of measuring circulating ACTH. in early diagnosis can only be estimated by relating the results from lung cancer patients to those of age and sex matched patients with chronic pulmonary disease and similar smoking habits.

In our study we have used as controls patients attending hospital with non-malignant pulmonary disease. We have taken as the upper limit of normal the upper limit of the range of values from these patients. In this way we have hoped to assess the frequency with which circulating ACTH. is raised due to secretion by tumour tissue.

METHODS

(a) Patients and Samples

134 patients attending the out-patient department or admitted to hospitals in Marburg and Glasgow during 1978 and 1979 were included in this study (Ratcliffe, Podmore, Stack *et al.*, 1981). The diagnosis of lung cancer was confirmed by bronchial or pleural biopsy, biopsy of enlarged lymph nodes or other metastases, sputum cytology or eventually at autopsy. Patients in whom the tumour was confined to one hemithorax and

ipsilateral mediastinal and cervical lymph nodes were considered to have localised disease. More widespread disease was classified as extensive. Tumours were divided into small and non-small cell groups according to the WHO classification by pathologists who were unaware of the clinical details and laboratory findings.

The controls were patients attending a Glasgow hospital with non-malignant pulmonary disease (Table 43). They were matched for age and sex with the Glasgow lung cancer patients. None of the patients or controls were taking corticosteroid drugs and none suffered from overt Cushing's syndrome. 81% of the Glasgow lung cancer patients were smokers compared with 52% of the controls.

10 ml. samples of venous blood were withdrawn between 9 and 10 a.m. into heparinised or plain bottles. These were immediately centrifuged and the serum or plasma snap frozen and stored under dry ice ($-20^{\circ}C$) until radio-immunoassay could be performed.

Laboratory Methods

Unextracted plasma and serum ACTH concentrations were measured using a double antibody radioimmunoassay. Antiserum against 1-39 human ACTH. was raised in rabbits and labelled 1-39 human ACTH. was used for iodination. The assay was validated by comparing ACTH. values so obtained with those found using a well-validated N-terminal ACTH. assay on extracted plasma (Ratcliffe and Edwards, 1971).

	Controls	Lung Cancer Patients	
		Unextracted method	Extracted method
Total number	30	113	21
Number of males	23	99	18
Mean age	62	63	66
Age range	45-85	46-78	53-82
Diagnosis:			
chronic bronchitis	17		
bronchopulmonary infection	7		
asthma	3		
miscellaneous	3		
Smokers	52%	81%	

Table 43. Clinical details of control and lung cancer patients in study of circulating immunoreactive hormones.

Extracted plasma ACTH. levels were measured using porous glass (Ratcliffe and Edwards, 1971). This selected against high molecular weight ACTH. components and C-terminal fragments of ACTH. so that the assay was relatively specific for 1-39 ACTH.. ACTH. was assayed as described by Rees, Cook, Kendall et al. (1971) using iodinated human 1-39 ACTH. as tracer and antiserum directed towards the biologically active 1-24 region of the molecule. This was standardised against natural human ACTH.. The limit of detection was 10 ng/l.

βMSH immunoactivity was assayed by the method of Gray and Ratcliffe (1979) standardised with synthetic human βMSH (Ciba) using an antiserum which cross reacts equally on a molar basis with human βMSH, βLPH and γ LPH. There was no cross reaction with ACTH. and its fragments, β endorphin or enkephalins. The limit of detection was 10 ng/l.

LPH immunoactivity was assayed by the method of Podmore, Wilson, Cowden et al. (1979) using iodinated βLPH and an antiserum which cross reacts equally on a molar basis with human β and γ LPH. There was no cross reaction with human βMSH, ACTH. and its fragments, β endorphin or enkephalins. The assay was standardised against purified human βLPH. The limit of detection was 80 ng/l.

RESULTS

It can be seen from Table 43 that the controls and patients were of similar ages and sex ratio. Two thirds of the controls suffered from chronic bronchitis or

asthma and seven were receiving treatment for pneumonia or pulmonary tuberculosis. The control values were all similar to the laboratory reference values.

When the unextracted method was used, serum levels were rather lower than plasma levels. This was shown by the difference in the range of ACTH. concentration in serum and plasma. Similarly 77% of serum concentrations of ACTH. but only 58% of plasma concentrations in all patients were less than 30 ng/l (Table 44).

When the extraction method was used, no patients had plasma ACTH. or β-MSH. concentrations above the control range and in only one patient was the concentration of LPH. raised (Table 45).

The Marburg group contributed a relatively large proportion of the small cell carcinoma patients in whom serum rather than plasma levels were measured. The overall incidence of elevated ACTH. levels was 7% for plasma and 17% for serum. For small cell carcinoma these values were 17% and 24% respectively and for non-small cell carcinoma 0% and 3%.

There was a higher proportion of elevated ACTH. concentrations in patients with extensive (32%) as opposed to limited (12%) small cell carcinoma. No such difference could be detected in the small numbers of non-small cell carcinoma with elevated ACTH. levels.

DISCUSSION

This investigation has demonstrated:

Lung Cancer	PLASMA			SERUM		
	Total No.	No. > control range	% > control range	Total No.	No.> control range	% > control range
Small cell						
Limited	6	0	0	25	3	12
Extensive	12	3	25	38	12	32
All cases	18	3	17	63	15	24
Non-small cell						
Limited	20	0	0	20	0	0
Extensive	6	0	0	10	1	10
All cases	26	0	0	30	1	3
All lung cancer cases	44	3	7	93	16	17

Table 44. Prevalence of raised total ACTH. levels in serum and plasma in patients with lung cancer. The control ranges were < 20-73 ng/l for plasma and < 20-50 ng/l for serum.

Lung Cancer	ACTH. ng/l			β-MSH. ng/l			LPH. ng/l		
	Range	No.	No.>control range	Range	No.	No.>control range	Range	No.	No.>control range
Small cell	10-34	7	0	13-51	7	0	80-256	7	0
Non-small cell	10-35	14	0	16-49	14	0	80-356	14	1
All cases	10-35	21	0	13-57	21	0	80-356	21	1

Table 45. Prevalence of elevated plasma levels of ACTH., β-MSH. and LPH. using extraction method. Control ranges were < 10-73 ng/l for ACTH., 14-62 ng/l for MSH. and < 80-341 ng/l for LPH.

1. A relatively low prevalence of raised serum and plasma ACTH. in lung cancer.
2. Elevation of circulating ACTH. predominantly in small cell carcinoma, especially where this was extensive.

Prevalence of raised ACTH. levels in plasma and serum

The prevalence of raised ACTH. levels in this study was similar to that of three other European groups (Gropp et al., 1980; Hansen et al., 1980; Torstensson, Thoren and Hall, 1980) but much less than that found by two American groups (Ayvazian et al., 1975 and Yalow, Eastridge, Higgins and Wolf, 1979; Wolfsen et al., 1979), (Table 42). Possible reasons for this discrepancy can be considered under four headings:

1. Samples
2. Laboratory methods
3. Patients
4. Definition of the upper limit of "normal".

Samples

Most workers have studied plasma levels. However Gropp et al. (1980) also measured some serum concentrations of ACTH.. Serum levels were also measured in some patients in the current study. As expected serum levels were rather lower both in normal and lung cancer patients. This may be due to the destruction of some ACTH. during the clotting process. As the other European workers measured plasma levels, this detail cannot explain the discrepancy between American and European results.

In our study samples were withdrawn between 9 and 10 a.m. Circulating ACTH. levels are known to be highest at between 8 and 10 a.m. and to be lower later in the day in normal subjects. Although the times of withdrawal of specimens are not clearly stated in some reports, Ayvazian et al. (1975) withdrew their specimens after 3 p.m. If time of day were the cause of the discrepancy, their results would be expected to be lower than those in this investigation.

Laboratory Methods

In all the studies ACTH. and other hormones were measured by a radioimmunoassay technique in which antibodies to the hormone were raised in animals. These antibodies react to a certain sequence of amino acids in the ACTH. molecule adjacent to the N terminal. However the specificity of the antiserum used in different laboratories is likely to have varied. Some antibodies may also react with C terminal elements in "big ACTH.", related peptide hormones (e.g. β-MSH. and LPH. in the ACTH. assay) and other serum proteins.

The specificity of the radioimmunoassay can be increased if the plasma is "extracted". This "extraction" method measures mainly physiological or 1-39 ACTH.. As this hormone comprises only 20-40% of ACTH. in lung tumours (Yesner, 1978) a lower prevalence of raised ACTH. concentrations would be expected when this method is used. This was indeed the finding of our own study (Table 45) but it also does not explain the discrepancy

with the American results. Indeed Wolfsen et al. (1979) found raised levels of ACTH. in 74% of lung cancer patients using the "extraction method". Their results were similar to those of Yalow et al. (1979) who studied unextracted plasma.

Patients

(a) Histology of the tumour

As the majority of lung tumours associated with overt Cushing's syndrome are small cell carcinomas, the prevalence of raised ACTH levels was compared in small and non-small cell carcinoma (Table 44). In this study and in those of Gropp et al. (1980) and Hansen et al. (1980) circulating ACTH. levels were raised in very similar proportions of small cell carcinoma patients but in the series of Ayvazian et al. (1975) the levels were raised in the only two small cell carcinoma cases. There were no small cell carcinomas in the surgical series of Yalow et al. (1979) as it was not current practice to operate on patients with this tumour.

The most striking discrepancy between the American and European results was found in non-small cell carcinoma. Whereas the New York workers found elevated ACTH. levels in 72% of cases, the prevalence was only 3% and 10% respectively in the current report and in the series of Gropp et al. (1980).

(b) Size of tumour

Ayvazian et al. (1975) calculated that either a tumour must be large or that it must have more active ACTH.

production than the pituitary gland if circulating ACTH. levels were to be elevated. Tumour size is only one facet of tumour stage which also reflects local and distant spread. If the tumours in the series of Wolfsen et al. (1979) and Yalow et al. (1979) were larger or more widespread than those in the other series, the higher prevalence of raised ACTH. levels would be explained. However the reverse is the case since Yalow et al. (1979) studied only surgical cases, most of whom would have stage I or stage 2 tumours. Moreover many of the patients of Wolfsen et al. (1979) were diagnosed on the basis of peripheral radiographic opacities. In contrast, in the current study and that of Gropp et al. (1980), the series were unselected and contained patients with extensive disease.

Definition of the upper limit of "normal"

The prevalence of raised levels of circulating ACTH. clearly depends on the upper limit of "normal". How this limit was defined by seven groups of workers is summarised in Table 42. Only Gropp et al. (1980) used age and sex matched controls. In our own study and in that of Torstensson et al. (1980) the reference value was derived by measuring circulating ACTH. concentrations in patients with benign pulmonary disease and in both these studies the age ranges of the lung cancer and control patients were similar.

Gewirtz et al. (1974) found ACTH. levels above an arbitrary reference value of 150 ng/l in 31% of patients

with chronic obstructive pulmonary disease. As a large proportion of lung cancer patients also suffer from this, their reference value was set too low as was that of Wolfsen et al. (1979). This error may explain much of the discrepancy between the American results and those of Torstensson et al. (1980) and the current investigation.

Predominance of small cell carcinoma in patients with raised circulating ACTH. levels

In this study, serum ACTH. levels were elevated in 24% small cell carcinoma but only 3% non-small cell carcinoma patients. This finding confirms previous descriptions of the association between elevated ACTH. levels in tissue and plasma or serum and small cell carcinoma.

In early reports of the ectopic ACTH. syndrome (Brown, 1928; Meador et al., 1962) all four patients with lung tumours had small cell carcinomas. Moreover, in a review of the literature, Broder (1979) found that 80/95 patients with this syndrome in whom histology had been recorded suffered from this histological type of tumour. In series of 185 (Azzopardi et al., 1970) and 280 (Rassam and Anderson, 1975) unselected lung cancer patients, the two patients with this syndrome both had small cell carcinoma. Moreover, Richardson, Greco, Oldham and Liddle (1978) estimated that this syndrome occurred in 7% of all patients who developed small cell carcinoma.

Others have confirmed that in patients with lung cancer who do not have overt Cushing's syndrome, elevated

ACTH. levels are more prevalent in small cell carcinoma (Gropp et al., 1980). Neither these workers nor Hansen et al. (1980a) found any relationship between ACTH. levels and the clinical stage of the disease. However our study did show that elevated circulating ACTH. levels in small cell carcinoma were more prevalent in patients with extensive disease.

If the number of tumour cells is a function of the extent of the tumour, this finding is compatible with the belief that in small cell carcinoma ectopic ACTH. originates from the tumour cells. Support for this belief also comes from the work of Bertagna, Nicholson, Sorenson et al. (1978) who found a linear relationship between the number of human small cell carcinoma cells during the first three days of cell culture and the concentration of ACTH. in the culture medium. A possible site of storage of this ectopic ACTH. is the secretory granules (Rees and Ratcliffe, 1974). Ultramicroscopic examination of these granules shows that they are similar to those found in typical peptide-secreting glands. Though present in small cell carcinoma, they were more numerous in ACTH.-rich bronchial carcinoids but were not found in non-small cell carcinoma.

There is thus some controversy as to whether ACTH. is actively produced in non-small cell carcinoma. Bloomfield, Holdaway, Corrin et al. (1977) found significant tissue levels in two patients with small cell carcinoma

but in none of six patients with other histological types. In contrast detectable levels of ACTH. were found in the majority of patients with operable non-small cell carcinoma by Yalow et al. (1979) although these workers later reported higher tissue levels of ACTH. in small cell carcinoma (Yalow, 1979b). However Ratcliffe and Podmore (1979) suggested that the ACTH. in non-small cell carcinoma could either indicate the presence of carcinoid or small cell tissue within such tumours or, in the case of levels $\leq$ 1 ng/g, retained circulating hormone or non-specific effects in the assay.

Is tumour tissue the only source of ectopic ACTH.? The finding of raised levels of plasma ACTH. in 31% of controls with chronic obstructive pulmonary disease led Gewirtz and Yalow (1974) to consider whether damaged normal lung could produce ACTH.. They failed to prove this in normal human lung tissue taken at operation and in normal lung tissue taken from a dog exposed to cigarette smoke. However they did find elevated levels of ACTH. in a similar dog in which precancerous epithelial changes of the type seen in heavy smokers and lung cancer patients were present. Moreover, Bloomfield et al. (1977) found that ACTH. concentrations from normal lung tissue taken from the same lobe or lung remote from the tumour correlated closely with ACTH. levels in the tumour itself. Hence it seems possible that cells throughout the lung of such patients secrete ACTH. They may do so as part of

the precancerous process or as a result of the development of neoplasm. Such cells could be the Kulchitsky cells which have been described in bronchial epithelium (Yesner, 1978). An alternative hypothesis is that the ACTH. is produced by secretory granules that have migrated from the primary tumour tissue.

ACTH. concentrations correlate closely with those of related hormones. Concentrations of ACTH, β-endorphin and LPH. rose in parallel in tissue cultures from small cell carcinoma (Bertagna et al., 1978). A close relationship between concentrations of ACTH. and those of LPH (Ratcliffe and Podmore, 1979) and β-MSH. (Gray and Ratcliffe, 1979) have been described. These hormones share a common hexapeptide sequence. It is now widely recognised that many different hormones can be produced in association with lung cancer, especially small cell carcinoma (Rees, Bloomfield, Rees et al., 1974).

Value of measuring circulating ACTH in clinical practice

The European results show that plasma ACTH. levels are raised in less than 30% of unselected small cell carcinoma patients and in a considerably lower proportion of non-small cell carcinoma patients. Thus this measurement is of no value in the screening of "at risk" populations or in the diagnosis of the individual patient.

The finding of a raised plasma ACTH level may be helpful in assessing the response to treatment. Elevated ACTH. concentrations fell to normal in all four small cell carcinoma patients treated with chemotherapy by

Hansen, Hammer and Hummer (1980). Three of these also had objective regression of tumour. Such a response may occur within 10 days of a course of chemotherapy (Gropp et al., 1980). Elevated pre-operative plasma ACTH. levels also fell in 12/21 patients undergoing resection of bronchial carcinoma (Yalow et al., 1979). Failure of elevated levels to fall in 9/21 appeared to strengthen the previous evidence that ectopic ACTH. may also arise from lung tissue which has been damaged or which has undergone precancerous change.

After an elevated ACTH. concentration has fallen to normal following treatment, a subsequent rise may indicate tumour recurrence and progression (Gropp et al., 1980). However failure of plasma ACTH. to rise by no means rules out the possibility of tumour recurrence (Hansen et al., 1980b). Finally pre-operative ACTH. concentrations gave no guide to prognosis in patients undergoing surgical resection of lung cancer (Yalow et al., 1979).

The finding of raised levels of circulating ACTH. and related hormones in lung cancer patients is of considerable research interest. However at present measurement of these hormones, either individually or collectively, is not likely to be of much value in the assessment and management of the individual patient or in the screening of populations at risk.

CONCLUSIONS

There is abundant evidence of a host defence against tumours which involves the activity of lymphocytes and macrophages. In lung cancer, tumour-associated antigens, antibodies and circulating immune complexes have all been described; lung cancer patients with active immunological reactions to antigens survive longer than those without.

Our studies have shown a higher prevalence of abnormal results in lung cancer patients than those found in controls. In general, the abnormalities have been less prevalent than those reported by other authors. This discrepancy seems to arise from their selection of unsuitable controls. Impairment of immunological tests is commoner in smokers, in patients with benign pulmonary disease and in older patients. Thus to assess the relevance of a particular test in lung cancer patients who are usually middle-aged or elderly, cigarette smokers and sufferers from chronic bronchitis, controls matched for age, sex, smoking habit and presence of benign pulmonary disease should be used.

This investigation has shown that abnormal immunological responses do occur even in patients with operable lung cancer. Recognition of new antigenic material (DNCB.) is impaired suggesting that there is a defect in the immunological surveillance mechanism. Moreover lymphocyte transformation by PPD. but not by PHA. or PWM. was depressed. This could be due to the action of PPD. stimulated suppressor cells on other lymphocytes.

Can specific autologous immunotherapy enhance the elimination of residual tumour cells in patients who have undergone resection of the bulk of the tumour? In the pilot trial, intradermal injection of irradiated autologous tumour cells and intradermal BCG. was followed by increased tuberculin reactivity and leukocytosis in the autograft group. This resulted in serious local ulceration, especially in strong tuberculin reactors, but there was no significant difference in clinical results between the autograft and non-autograft groups of patients, both of whom were treated with radiotherapy. In the subsequent main trial, the overall effect of postoperative serial percutaneous injections of autologous irradiated tumour cells and BCG. was to produce a prolonged rise in tuberculin reactivity but only a modest improvement in clinical results. There was some evidence that this treatment improved the survival and duration of freedom from tumour recurrence of patients with stage I tumours and those who became sensitised to DNCB. before operation. Hence in these patients, specific autologous immunotherapy may have a place as an adjuvant to surgery.

In an assessment of the value of measuring circulating antigenic markers in the diagnosis of lung cancer, it was found that the prevalences of elevated levels of both ACTH. and CEA. in unselected lung cancer patients were much lower than those found by some other authors. This was due to the selection as a reference value of the upper limit of values recorded from age and sex matched

controls with benign pulmonary disease rather than that of normal healthy controls. Levels of both of these markers were higher in patients with extensive disease and of ACTH. in patients with small cell carcinoma. However the prevalence of elevated circulating antigenic markers, either individually or in combination, was not high enough for these measurements to be of value in the screening of populations for lung cancer or its diagnosis in patients. Where initial levels of these markers are elevated, serial measurements may be of value in monitoring the progress of the disease.

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